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(54) Title: **PORPHYROMONAS GINGIVALIS ARGinine-SPECIFIC PROTEINASE CODING SEQUENCES**

(57) Abstract

Provided herein is a nucleotide sequence encoding an Arg-specific gingipain named gingipain-1 isolated from Porphyromonas gingivalis, having an apparent molecular mass of 50 kDa as estimated by SDS gel electrophoresis and an apparent molecular mass of 44 kDa as estimated by gel filtration chromatography. Gingipain-1 has amidolytic and proteolytic activity for cleavage after arginine residues and has no amidolytic and/or proteolytic activity for cleavage after lysine residues. Its activity is inhibited cysteine protease group-specific inhibitors and chelating agents. It is stabilized by Calcium and stimulated by glycine-containing peptides and glycine analogues.

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**PORPHYROMONAS GINGIVALIS ARGININE-SPECIFIC PROTEINASE
CODING SEQUENCES**

This invention was made, at least in part, with funding from
5 the National Institutes of Health (Grant Nos. DE 09761, HL 26148
and HL 37090). Accordingly, the United States Government may
have certain rights in this invention.

FIELD OF THE INVENTION

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The field of this invention is bacterial proteases, more particularly those of Porphyromonas gingivalis, most particularly the arginine-specific protease termed Arg-gingipain herein and the nucleotide sequences encoding same.

15

BACKGROUND OF THE INVENTION

Porphyromonas gingivalis (formerly Bacteroides gingivalis) is an obligately anaerobic bacterium which is implicated in periodontal disease. P. gingivalis produces proteolytic enzymes in relatively large quantities; these proteinases are recognized as important virulence factors. A number of physiologically significant proteins, including collagen, fibronectin, immunoglobulins, complement factors C3, C4, C5, and B, lysozyme, iron-binding proteins, plasma proteinase inhibitors, fibrin and fibrinogen, and key factors of the plasma coagulation cascade system, are hydrolyzed by proteinases from this microorganism. Such broad proteolytic activity may play a major role in the evasion of host defense mechanisms and the destruction of gingival connective tissue associated with progressive periodontitis (Saglie et al. (1988) *J. Periodontol.* 59, 259-265).

There are conflicting data as to the number and types of proteinases produced by P. gingivalis. In the past, proteolytic activities of P. gingivalis were classified into two groups; those enzymes which specifically degraded collagen and the 5 general "trypsin-like" proteinases which appeared to be responsible for other proteolytic activity. Trypsin (and trypsin-like proteases) cleaves after arginine or lysine in the substrates (See, e.g. Lehninger A. L. (1982), Principles of Biochemistry, Worth Publishing, Inc., New York). Although many 10 attempts have been made to separate one of these trypsin-like proteinases, Chen et al. (1992) *J. Biol. Chem.* 267, 18896-18901 reported the first rigorous purification and biochemical and enzymological characterization for an Arginine-specific P. gingivalis protease.

15

This application reports the purification of 50 kDa and high molecular weight trypsin-like, thiol-activated proteinases of P. gingivalis and nucleotide sequences encoding same.

20 SUMMARY OF THE INVENTION

An object of the present invention is to provide a nucleotide sequence encoding a low molecular weight Arg-gingipain, termed Arg-gingipain-1 (or gingipain-1), herein, said 25 gingipain-1 having an apparent molecular mass of 50 kDa as estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and an apparent molecular mass of 44 kDa as estimated by gel filtration chromatography, said gingipain-1 having amidolytic and proteolytic activity for cleavage after 30 arginine residues and having no amidolytic and/or proteolytic activity for cleavage after lysine residues, wherein the amidolytic and/or proteolytic activity is inhibited by cysteine protease group-specific inhibitors including iodoacetamide, iodoacetic acid, N-ethylmaleimide, leupeptin, antipain, trans-epoxysuccinyl-L-leucylamido-(4-guanidine)butane, TLCK, TPCK, p- 35 aminobenzamidine, N-chlorosuccinamide, and chelating agents including EDTA and EGTA, wherein the amidolytic and/or

proteolytic activity of said gingipain-1 is not sensitive to inhibition by human cystatin C, α_2 -macroglobulin, α_1 -proteinase inhibitor, antithrombin III, α_2 -antiplasmin, serine protease group-specific inhibitors including diisopropylfluorophosphate, phenylmethyl sulfonylfluoride and 3,4-diisochlorocoumarin, and wherein the amidolytic and/or proteolytic activities of gingipain-1 are stabilized by Ca^{2+} and wherein the amidolytic and/or proteolytic activities of said gingipain-1 are stimulated by glycine-containing peptides and glycine analogues. In a specifically exemplified gingipain-1 protein, the protein is characterized by an N-terminal amino acid sequence as given in SEQ ID NO:1 Tyr-Thr-Pro-Val-Glu-Glu-Lys-Gln-Asn-Gly-Arg-Met-Ile-Val-Ile-Val-Ala-Lys-Lys-Tyr-Glu-Gly-Asp-Ile-Lys-Asp-Phe-Val-Asp-Trp-Lys-Asn-Gln-Arg-Gly-Leu-Thr-Lys-Xaa-Val-Lys-Xaa-Ala) and by a C-terminal amino acid sequence as given in SEQ ID NO:6 (Glu-Leu-Leu-Arg).

A further object of this invention is a nucleotide sequence encoding a high molecular weight form of Arg-gingipain, termed Arg-gingipain-2 herein, which comprises a proteolytic component essentially as described hereinabove and at least one hemagglutinin component.

As specifically exemplified, the encoded Arg-gingipain-hemagglutinin complex is transcribed as a prepolypeptide, with the amino acid sequence as given in SEQ ID NO:11 from amino acid 1-1704. The encoded mature high molecular weight Arg-gingipain protein has a protease component having a complete deduced amino acid sequence as given in SEQ ID NO:11 from amino acid 228 through amino acid 719. An alternative protease component amino acid sequence is given in SEQ ID NO:4, amino acids 1-510. Arg-gingipain-2 further comprises at least one hemagglutinin component. The hemagglutinin components which are found associated with the 50 kDa Arg-specific proteolytic component are 44 kDa, 27 kDa and 17 kDa, and have amino acid sequences as given in SEQ ID NO:11, from 720 to 1091, from 1092 to 1429 and from 1430 to 1704, respectively.

It is an additional object of the invention to provide nucleic acid molecules for the recombinant production of an Arg-gingipain. Substantially pure recombinant Arg-gingipain-1 protein can be prepared after expression of the nucleotide sequences encoding Arg-gingipain in a heterologous host cell using the methods disclosed herein. Said substantially pure Arg-gingipain-1 exhibits amidolytic and/or proteolytic activity with specificity for cleavage after arginine, but exhibits no amidolytic and/or proteolytic activity with specificity for cleavage after lysine residues. The purification method exemplified herein comprises the steps of precipitating extracellular protein from cell-free culture supernatant of Porphyromonas gingivalis with ammonium sulfate (90% w/v saturation), fractionating the precipitated proteins by gel filtration, further fractionating by anion exchange chromatography those proteins in the fractions from gel filtration with the highest specific activity for amidolytic activity as measured with Benzoyl-L-arginyl-p-nitroanilide and collecting those proteins which were not bound to the anion exchange column, and fractionating those proteins by FPLC over a cation exchange column (MonoS HR5/5, Pharmacia, Piscataway, NJ) and finally separating gingipain-1 from lysine-specific proteolytic/amidolytic protein(s) by affinity chromatography over L-arginyl-agarose. Preferably the P. gingivalis used is strain H66, and preferably the culture is grown to early stationary phase. Arg-gingipain-1 can also be purified from cells using appropriate modifications of the foregoing procedures (cells must be disrupted, e.g., by lysis in a French pressure cell). Preferably the gel filtration step is carried out using Sephadex G-150, the anion exchange chromatography step is carried out using diethylaminoethyl (DEAE)-cellulose, the FPLC step is carried out using Mono S, and the affinity chromatography is carried out using L-arginyl-Sepharose 4B.

It is a further object of this invention to provide recombinant polynucleotides (e.g., a recombinant DNA molecule) comprising a nucleotide sequence encoding an Arg-gingipain

protein, preferably having an amino acid sequence as given in SEQ ID NO:11 from amino acid 228 through amino acid 719 or having an amino acid sequence as given in SEQ ID NO:4, amino acids 1 through 510. As specifically exemplified herein, the nucleotide sequence encoding a mature Arg-gingipain protease is given in SEQ ID NO:10, nucleotides 1630 through 3105, or SEQ ID NO:3 from nucleotides 1630 through 3105. The skilled artisan will understand that the amino acid sequence of the exemplified gingipain protein can be used to identify and isolate additional, nonexemplified nucleotide sequences which will encode a functional protein of the same amino acid sequence as given in SEQ ID NO:4 from amino acid 1 through amino acid 510 or an amino acid sequence of greater than 90% identity and having equivalent biological activity. The skilled artisan understands that it may be desirable to express the Arg-gingipain as a secreted protein; if so, he knows how to modify the exemplified coding sequence for the "mature" gingipain-2 by adding a nucleotide sequence encoding a signal peptide appropriate to the host in which the sequence is expressed. When it is desired that the sequence encoding an Arg-gingipain protein be expressed, then the skilled artisan will operably link transcription and translational control regulatory sequences to the coding sequences, with the choice of the regulatory sequences being determined by the host in which the coding sequence is to be expressed. With respect to a recombinant DNA molecule carrying an Arg-gingipain coding sequence, the skilled artisan will choose a vector (such as a plasmid or a viral vector) which can be introduced into and which can replicate in the host cell. The host cell can be a bacterium, preferably Escherichia coli, or a yeast or mammalian cell.

Also provided is a specific exemplification of a nucleotide sequence encoding an Arg-gingipain, including low molecular weight gingipain-1 protease component and the protease component of high molecular weight gingipain and its associated hemagglutinin components. These components are processed from a prepolypeptide. As specifically exemplified, the coding

sequence, from nucleotide 949 to nucleotide 6063 in SEQ ID NO:10, including the stop codon, encodes a prepolypeptide having an amino acid sequence as given in SEQ ID NO:11. The prepolypeptide is encoded by a nucleotide sequence as given in SEQ ID NO:10 from 5 nucleotide 949 to 6063. The mature protease molecule is encoded at nucleotides 1630 through 3105 in SEQ ID NO:10. The mature Arg-specific proteolytic component has an amino acid sequence as given in SEQ ID NO:11 from 228-719, and the hemagglutin component has an amino acid sequence as in SEQ ID NO:11 from 720-1091, from 10 1092 to 1429 or from 1430 to 1704.

In another embodiment, recombinant polynucleotides which encode an Arg-gingipain, including, e.g., protein fusions or deletions, as well as expression systems are provided. 15 Expression systems are defined as polynucleotides which, when transformed into an appropriate host cell, can express a proteinase. The recombinant polynucleotides possess a nucleotide sequence which is substantially similar to a natural Arg-gingipain-encoding polynucleotide or a fragment thereof.

20 The polynucleotides include RNA, cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified or contain non-natural or derivatized nucleotide bases. DNA is preferred. Recombinant polynucleotides comprising sequences otherwise not naturally occurring are also provided by this invention, as are alterations of a wild type proteinase sequence, including but not limited to deletion, insertion, substitution of one or more nucleotides or by fusion to other polynucleotide 25 sequences.

30 The present invention also provides for fusion polypeptides comprising an Arg-gingipain. Homologous polypeptides may be fusions between two or more proteinase sequences or between the sequences of a proteinase and a related protein. Likewise, heterologous fusions may be constructed which would exhibit a 35 combination of properties or activities of the proteins from

which they are derived. Fusion partners include but are not limited to immunoglobulins, ubiquitin bacterial β -galactosidase, trpE, protein A, β -lactamase, alpha amylase, alcohol dehydrogenase and yeast alpha mating factor, (Godowski et al. 5 (1988) *Science*, 241, 812-816). Fusion proteins will typically be made by recombinant methods but may be chemically synthesized.

Compositions and immunogenic preparations including but not limited to vaccines, comprising recombinant Arg-gingipain derived 10 from P. gingivalis and a suitable carrier therefor are provided. Such vaccines are useful, for example, in immunizing an animal, including humans, against inflammatory response and tissue damage caused by P. gingivalis in periodontal disease. The vaccine preparations comprise an immunogenic amount of a proteinase or 15 an immunogenic fragment or subunit thereof. Such vaccines may comprise one or more Arg-gingipain proteinases, or an Arg-gingipain in combination with another protein or other immunogen. By "immunogenic amount" is meant an amount capable of eliciting 20 the production of antibodies directed against one or more Arg-gingipains in an individual to which the vaccine has been administered.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the composite physical map of an Arg-gingipain locus. The first codon of the mature Arg-gingipain proteolytic component is indicated. Only major restriction sites employed in cloning are indicated: B, *Bam*HI; P, *Pst*I; S, *Sma*I; A, Asp 718; Pv, *Pvu*II; H, *Hind*III. The four arginine cleavage sites (R227, R719, R1091 and R1429) are each indicated with an asterisk (*). The three residues forming the active site (C412, H438 and N669, respectively) are also shown.

Figure 2 is a protein matrix plot, which presents analysis 35 of regions of similarity between hemagglutinin domains using Pustell Protein Matrix from MacVector, Release 4.0. The complete prepolypeptide sequence (SEQ ID NO:11) was used as X-axis and Y-

axis. The perfect diagonal row is the line of identity, whereas structure in the pattern near that diagonal corresponds to internal repeats. The four different domains are represented (Arg-gingipain protease, 44 kDa hemagglutinin, 17 kDa hemagglutinin and 27 kDa hemagglutinin). Four regions of high homology are identified. The main homologies between hemagglutinin domains is shown in detail in Table 4.

DETAILED DESCRIPTION OF THE INVENTION

10

Abbreviations used herein for amino acids are standard in the art: X or Xaa represents an amino acid residue that has not yet been identified but may be any amino acid residue including but not limited to phosphorylated tyrosine, threonine or serine, 15 as well as cysteine or a glycosylated amino acid residue. The abbreviations for amino acid residues as used herein are as follows: A, Ala, alanine; V, Val, valine; L, Leu, leucine; I, Ile, isoleucine; P, Pro, proline; F, Phe, phenylalanine; W, Trp, tryptophan; M, Met, methionine; G, Gly, glycine; S, Ser, serine; 20 T, Thr, threonine; C, Cys, cysteine; Y, Tyr, tyrosine; N, Asn, asparagine; Q, Gln, glutamine; D, Asp, aspartic acid; E, Glu, glutamic acid; K, Lys, lysine; R, Arg, arginine; and H, His, histidine. Other abbreviations used herein include Bz, benzoyl; Cbz, carboxybenzoyl; pNA, p-nitroanilide; MeO, methoxy; Suc, 25 succinyl; OR, ornithyl; Pip, pipocolyl; SDS, sodium dodecyl sulfate; TLCK, tosyl-L-lysine chloromethyl ketone; TPCK, tosyl-L-phenylalanine chloromethyl ketone; S-2238, D-Phe-Pip-Arg-pNA, S-2222, Bz-Ile-Glu-(γ -OR)-Gly-pNA; S-2288, D-Ile-Pro-Arg-pNA; S-2251, D-Val-Leu-Lys-pNA; Bis-Tris, 2-[bis(2-hydroxyethyl)amino]-30 2-(hydroxymethyl)-propane-1,3-diol; FPLC, fast protein liquid chromatography; HPLC, high performance liquid chromatography; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; EGTA, [ethylene-bis(oxyethylene-nitrile)tetraacetic acid; EDTA, ethylenediamine-tetraacetic acid; Z-L-Lys-pNa, Z-L-Lysine-p-Nitroanilide; HMW, high molecular weight.

Arg-gingipain is the term given to a P. gingivalis enzyme with specificity for proteolytic and/or amidolytic activity for cleavage of an amide bond, in which L-arginine contributes the carboxyl group. The Arg-gingipains described herein have identifying characteristics of cysteine dependence, inhibition response as described, Ca^{2+} - stabilization and glycine stimulation. Particular forms of Arg-gingipain are distinguished by their apparent molecular masses of the mature proteins (as measured without boiling before SDS-PAGE). Arg-gingipains of the present invention have no amidolytic or proteolytic activity for amide bonds in which L-lysine contributes the -COOH moiety.

Arg-gingipain-1 is the name given herein to a protein characterized as having a molecular mass of 50 kDa as measured by SDS-PAGE and 44 kDa as measured by gel filtration over Sephadex G-150, having amidolytic and/or proteolytic activity for substrates having L-Arg in the P₁ position, i.e. on the N-terminal side of the peptide bond to be hydrolyzed but having no activity against corresponding lysine-containing substrates being dependent on cysteine (or other thiol groups for full activity), having sensitivity to cysteine protease group-specific inhibitors including iodoacetamide, iodoacetic acid, and N-methylmaleimide, leupeptin, antipain, trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane, TLCK, TPCK, p-aminobenzamidine, N-chlorosuccinamide, and chelating agents including EDTA and EGTA, but being resistant to inhibition by human cystatin C, α_2 -macroglobulin, α_1 -proteinase inhibitor, antithrombin III, α_2 -antiplasmin, serine protease group-specific inhibitors including diisopropylfluorophosphate, phenylmethyl sulfonylfluoride and 3,4-diisochlorocoumarin, and wherein the amidolytic and/or proteolytic activities of gingipain-1 are stabilized by Ca^{2+} and wherein the amidolytic and/or proteolytic activities of said gingipain-1 are stimulated by glycine-containing peptides and glycine analogues.

35

An exemplified Arg-gingipain described and termed Arg-gingipain-2 herein exists in the native form in a high molecular

5 weight form, having an apparent molecular mass of 95 kDa as determined by SDS-PAGE, without boiling of samples. When boiled, the high molecular weight form appears to dissociate into components of 50 kDa, 43 kDa, 27 kDa and 17 kDa. Arg-gingipain-2 is the name given to the 50 kDa, enzymatically active component of the high molecular weight complex.

10 The complete amino acid sequence of an exemplified mature Arg-gingipain is given in SEQ ID NO:11, from amino acid 228 through amino acid 719. A second possible exemplary amino acid sequence is given in SEQ ID NO:4, amino acids 1 through 510. In nature these proteins are produced by the archebacterium Porphyromonas gingivalis; it can be purified from cells or from culture supernatant or as a recombinant expression product using 15 the methods provided herein. Without wishing to be bound by any theory, it is proposed that these sequences correspond to Arg-gingipain-2.

20 As used herein with respect to Arg-gingipain-1, a substantially pure Arg-gingipain preparation means that there is only one protein band visible after silver-staining an SDS polyacrylamide gel run with the preparation, and the only amidolytic and/or proteolytic activities are those with specificity for L-arginine in the P₁ position relative to the 25 bond cleaved. A substantially pure high molecular weight Arg-gingipain preparation has only one band (95 kDa) on SDS-PAGE (sample not boiled) or four bands (50 kDa, 43 kDa, 27 kDa, 17 kDa; sample boiled). No amidolytic or proteolytic activity for substrates with lysine in the P₁ position is evident in a 30 substantially pure high molecular weight or Arg-gingipain-2 preparation. Furthermore, a substantially pure preparation of Arg-gingipain has been separated from components with which it occurs in nature. Substantially pure Arg-gingipain is substantially free of naturally associated components when 35 separated from the native contaminants which accompany them in their natural state. Thus, Arg-gingipain that is chemically synthesized or recombinantly synthesized in a cellular system

different from the cell from which it naturally originates will be substantially free from its naturally associated components. Techniques for synthesis of polypeptides are described, for example, in Merrifield (1963) *J. Amer. Chem. Soc.*, 85, 2149-2156.

5

A chemically synthesized Arg-gingipain protein is considered an "isolated" polypeptide, as is an Arg-gingipain produced as an expression product of an isolated proteinase-encoding polynucleotide which is part of an expression vector (i.e., a "recombinant proteinase"), even if expressed in a homologous cell type.

Recombinant Arg-gingipain-1, Arg-gingipain-2 and HMW Arg-gingipain can be obtained by culturing host cells transformed with the recombinant polynucleotides comprising nucleotide sequences encoding an Arg-gingipain as described herein under conditions suitable to attain expression of the proteinase-encoding sequence.

20

Example 1 below and Chen et al. (1992) supra describe the purification of Arg-gingipain-1 and HMW Arg-gingipain from P. gingivalis culture supernatant, i.e., from a natural source. Various methods for the isolation of an Arg-gingipain from other biological material, such as from nonexemplified strains of P. gingivalis or from cells transformed with recombinant polynucleotides encoding such proteins, may be accomplished by methods known in the art. Various methods of protein purification are known in the art, including those described, e.g., in Guide to Protein Purification, ed. Deutscher, Vol. 182 of Methods in Enzymology (Academic Press, Inc.: San Diego, 1990) and Scopes, Protein Purification: Principles and Practice (Springer-Verlag: New York, 1982).

25

Chromatography over Sephadex G-150 yielded four peaks with Bz-L-Arg-pNA-hydrolyzing activity. In each of these fractions, the hydrolytic activity was dependent on cysteine and enhanced many-fold by the addition of glycyl-glycine or glycine amide.

30

Antibody specific for Arg-gingipain-1 immunoprecipitates proteinase from all four Sephadex G-150 peaks. Without wishing to be bound by any particular theory, it is postulated that the four-peak Bz-L-Arg-pNA-amidolytic profile is an anomaly resulting 5 from the binding of gingipain-1 to membrane or nucleic acid fragments. Alternatively, those peaks containing higher molecular weight protein may contain partially processed gingipain-1 precursors. Although the purification of gingipain-1 as exemplified is from extracellular protein, it can also be 10 purified from the bacterial cells.

Further analysis (see Example 1) of the high molecular weight fractions containing Arg-specific amidolytic and proteolytic activity revealed that Arg-gingipain-2 (50 kDa) 15 occurred non-covalently bound to proteins of 44 kDa, 27 kDa and 17. kDa, which have hemagglutinin activity. The empirically determined N-terminal amino acid sequence of the complexed 44 kDa protein corresponds to amino acids 720-736 of SEQ ID NO:11.

20 Arg-Gingipain-1 was further purified from the Sephadex G-150 Peak 4 protein mixture by further steps of anion exchange chromatography over DEAE-cellulose and two runs over Mono S FPLC. Arg-gingipain-1 recovery was markedly reduced if an affinity chromatography step (L-Arginyl-Sepharose 4B) was used to remove 25 trace amounts of a contaminating proteinase with specificity for cleavage after lysine residues.

Purified Arg-gingipain-1 exhibits an apparent molecular mass 30 of about 50 KDa as determined by SDS-polyacrylamide gel electrophoresis. The size estimate obtained by gel filtration on Superose 12 (Pharmacia, Piscataway, NJ) is 44 kDa. Amino-terminal sequence analysis through 43 residues gave a unique structure which showed no homology with any other proteins, based 35 on a comparison in the protein NBRS data base, release 39.0. The sequence obtained is as follows:

Tyr-Thr-Pro-Val-Glu-Glu-Lys-Gln-Asn-Gly-Arg-Met-Ile-Val-Ile-Val-Ala-Lys-Lys-Tyr-Glu-Gly-Asp-Ile-Lys-Asp-Phe-Val-Asp-Trp-Lys-Asn-

Gln-Arg-Gly-Leu-Thr-Lys-Xaa-Val-Lys-Xaa-Ala (SEQ ID NO:1).

The C-terminal amino acid sequence of the gingipain-1 (major form recognized in zymography SDS-PAGE, 0.1% gelatin in gel), was found to be Glu-Leu-Leu-Arg. (SEQ ID NO:5). This corresponds to the amino acids 716-719 in SEQ ID NO:4 and nucleotides 3094-3105 in SEQ ID NO:3. This is consistent with the model for autoproteolytic processing of the precursor polyprotein to produce the mature 50 kDa gingipain-1 protein.

Comparison of SEQ ID NO:1 with SEQ ID NO:4 and 11 shows differences at amino acids 37-38 of the mature Arg-gingipain. Without wishing to be bound by any theory, it is proposed that SEQ ID NO:3 (or SEQ ID NO:10) comprises the coding sequence for Arg-gingipain-2, the enzymatically active component of the high molecular weight form of Arg-gingipain. This is consistent with the observation that there are at least two genes with substantial nucleic acid homology to the Arg-gingipain-specific probe.

The enzymatic activity of Arg-gingipain-1 is stimulated by glycine and glycine-containing compounds. In the absence of a glycine-containing compound, the enzyme has essentially the same amidolytic activity in the pH range 7.5-9.0. However, in the presence of glycyl-glycine, e.g., substantial sharpening of the pH range for activity is observed, with the optimum being between pH 7.4 and 8.0. Preliminary kinetic data indicate that the effect of glycine and glycine analogues is to raise both k_{cat} and K_m equally so that the k_{cat}/K_m ratio does not change. It is therefore likely that these compounds bind to the enzyme and/or substrate after an enzyme-substrate complex has already formed. The high molecular weight form is stimulated only about half as much by glycine compounds.

Arg-gingipain-1 requires cysteine for full amidolytic activity, and, although it is stimulated by other thiol-containing compounds, the effect was less pronounced. Cysteine and

cysteamine are most efficient, presumably because they perform the dual roles of reducing agents and glycine analogues.

5 The amidolytic activity of Arg-gingipain-1 is inhibited by a number of -SH blocking group reagents, oxidants, Ca^{2+} chelating agents, and Zn^{2+} . The effect of chelating agents EDTA and EGTA was reversed completely by the addition of excess Ca^{2+} , whereas in the case of Zn^{2+} , it was necessary to add o-phenanthroline prior to Ca^{2+} .

10

Typical serine proteinase group-specific inhibitors have no effect on enzyme activity, and it is likely that inhibition by both TLCK and TPCK was caused by reaction with an essential cysteine residue in the enzyme, a known property of chloromethyl ketone derivatives. Significantly, Arg-gingipain-1 was inhibited by such cysteine proteinase inhibitors as trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane, leupeptin and antipain. Although the reactions were not stoichiometric, the inhibition was concentration-dependent. However, human cystatin C, an inhibitor of mammalian and plant cysteine proteinases, does not inhibit Arg-gingipain-1, nor did any of the trypsin-specific inhibitors from human plasma, including α_2 -macroglobulin, α_1 -proteinase inhibitor, antithrombin III, and α_2 -antiplasmin. Indeed, preliminary investigations actually suggested that the inhibitor in each case was being inactivated by Arg-gingipain-1.

30 Calcium ion stabilizes Arg-gingipain-1 without directly affecting activity. With Ca^{2+} present the enzyme is stable in the pH range between 4.5 and 7.5 for several days at 4°C. However, below pH 4.0 or in the absence of Ca^{2+} , enzyme activity is quickly lost. At 37°C Ca^{2+} considerably increases stability, although activity is lost more rapidly than at the lower temperature. At -20°C Arg-gingipain-1 is stable for several months. During lyophilization, however, it irreversibly loses 35 more than 90% of its catalytic activity.

The amidolytic activity of the purified Arg-gingipain-1 on synthetic peptide substrates was limited to substrates with a P₁-Arg residue. Even then Arg-gingipain-1 had significantly different turnover rates on individual substrates, being most effective against S-2238 (D-Phe-Pip-Arg-pNA) and S-2222 (Bz-Ile-Glu-(γ -OR)-Gly Arg-pNA). Lesser, comparable activity was observed using S2288 (D-Ile-Pro-Arg-pNA) and Bz-Arg-pNA. D-Val-Leu-Lys-pNA (S-2251), Suc-Ala-Ala-Ala-pNA, MeO-Suc-Ala-Ala--Pro-Val-pNA, Suc-Ala-Ala-Pro-Phe-pNA, Gly-Pro-pNA and Cbz-Phe-Leu-Glu-pNA had essentially no substrate activity. This narrow specificity was confirmed by examination of the cleavage products after incubation with the insulin B chain or mellitin; it was found that cleavage occurred specifically after only Arg residues, but not after Lys or any other amino acids unless the last affinity chromatography step over L-Arginine-Sepharose 4B was omitted.

Because progressive periodontitis is characterized by tissue degradation, collagen destruction and a strong inflammatory response, and because *P. gingivalis* was known to exhibit complement-hydrolyzing activity, purified Arg-gingipain-1 was tested for proteinase activity using purified human complement C3 and C5 as substrates (See Wingrove et al. (1992) J. Biol. Chem. 267: 18902-18907). Low molecular weight Arg-gingipain selectively cleaved the α -chain, generating what initially appeared to be the α' -chain of C3b. Further breakdown fragments of the C3 α' -chain were observed and a decreasing intensity of the α' -band suggested that degradation continued. Visual evidence suggested that the C3 β -chain is resistant to this proteinase. Attempts to demonstrate C3a biological activity in the C3 digestion mixture were unsuccessful, and the C3a-like fragment released from the α -chain was extensively degraded by Arg-gingipain-1.

Human C5 was also digested by Arg-gingipain-1, with initial cleavage specific for the C5 α -chain, as in the case of C3. The α -1 (86 kDa) and the α -2 (30 kDa) fragments were the first

polypeptides to be formed from cleavage of C5 by gingipain-1, and they equal the molecular weight of the intact α -chain, a fragment in the size range of C5a was observed. C5a is more resistant to the Arg-gingipain-1 than C3a, and functional C5a may accumulate without further appreciable degradation. C5a biological activity was detected after digestion of human C5 with Arg-gingipain-1. Characteristic morphologic changes in human neutrophils, known as polarization, were scored by counting deformed cells relative to normally rounded cells.

10

To test for in vivo biological activity, the purified low molecular weight Arg-gingipain enzyme was injected into guinea pig skin. It induced vascular permeability enhancement at concentrations greater than 10^{-8} M in dose-dependent and proteolytic activity dependent manners. Vascular permeability enhancement activity was not inhibited by diphenhydramine (an antihistamine), and the activity was enhanced by SQ 20,881 (angiotensin-converting enzyme inhibitor). The vascular permeability enhancement by Arg-gingipain-1 was inhibited by soybean trypsin inhibitor (SBTI) at a concentration of 10^{-5} M, a concentration at which SBTI did not inhibit enzymatic activity, as measured with Bz-L-Arg-pNA and azocasein as the substrates.

Human plasma or guinea pig plasma treated with Arg-gingipain-1 (10^{-8} to 10^{-6} M) induced vascular permeability enhancement in the guinea pig skin assay. Vascular permeability enhancement by Arg-gingipain-1 treated plasma was increased by addition of 1,10-phenanthroline (kinase inhibitor, chelating agent for Zn ions) to a final concentration of 1 mM. Vascular permeability enhancement by Arg-gingipain-1 treated plasmas was markedly reduced when plasmas deficient in Hageman factor, prekallikrein or high molecular weight kininogen were used. These results indicate that vascular permeabilizing enhancement by Arg-gingipain-1 acts via activation of Hageman factor and the subsequent release of bradykinin from high molecular weight kininogen by kallikrein.

Intradermal injection of Arg-gingipain-1 in the guinea pig also resulted in neutrophil accumulation at the site of injection, an activity which was dependent on proteolytic activity.

5

The foregoing results demonstrate the ability of Arg-gingipain to elicit inflammatory responses in a guinea pig animal model.

10 Recombinant Arg-gingipain is useful in methods of identifying agents that modulate Arg-gingipain proteinase activity, whether by acting on the proteinase itself or preventing the interaction of a proteinase with a protein in gingival area, such as C3 or C5. One such method comprises the
15 steps of incubating a proteinase with a putative therapeutic, i.e., Arg-gingipain-inhibiting, agent; determining the activity of the proteinase incubated with the agent; and comparing the activity obtained in step with the activity of a control sample of proteinase that has not been incubated with the agent.

20

SDS-PAGE analysis (without boiling) of the purified high molecular weight form of Arg-gingipain revealed a single band of apparent molecular mass of 95 kDa. This estimate was confirmed by analytical chromatography over a TSK 3000SW gel filtration column. When the enzyme preparation was boiled before SDS-PAGE, however, bands of apparent molecular masses of approximately 50 kDa, 44 kDa, 27 kDa and 17 kDa were observed. These bands were not generated by treatments at temperatures below boiling, by reducing agents or detergents. It was concluded that the 95 kDa band was the result of strong non-covalent binding between the lower molecular weight proteins.

30
35 The 50 kDa proteolytic component of the high molecular weight Arg-gingipain was characterized with respect to N-terminal amino acid sequence over 22 amino acids. The sequence was identical to the first 22 amino acids of the 50 kDa, low molecular weight Arg-gingipain-1. Characterization of the high

5 molecular weight Arg-gingipain activity showed the same dependence on cysteine (or other thiols) and the same spectrum of response to potential inhibitors. Although the high molecular weight Arg-gingipain was stimulated by glycine compounds, the response was only about half that observed for the low molecular weight form.

The primary structure of the NH₂-terminus of low molecular weight Arg-gingipain determined by direct amino acid sequencing. 10 (SEQ ID NO:1) was used to prepare a mixture of synthetic primer oligonucleotides GIN-1-32 (SEQ ID NO:6) coding for amino acids 2 to 8 of the mature protein and primer GIN-2-30 (SEQ ID NO:7) coding for amino acids 25-32 of the mature protein. These primers were used in PCR on P. gingivalis DNA. A single 105-base 15 pair product (P105) resulted. This was cloned into pCR-Script™SK(-) (Stratagene) and sequenced. Sequence analysis of P105 generated 49 nucleotides from an Arg-gingipain coding sequence. On the basis of the sequence of P105, another primer 20 (GIN-8S-48) SEQ ID NO:8 corresponding to the coding strand of the partial Arg-gingipain gene (48-mers) was synthesized in order to screen the λDASH DNA library using a ³²P-labeled GIN-8S-48 probe. A partial sequence of the Arg-gingipain gene (nucleotides 1-3159, 25 SEQ ID NO:3) was determined by screening the P. gingivalis DNA library using ³²P-labeled hybridization GIN-8S-48 probe (SEQ ID NO:8). From a total of 2x10⁵ independent plaques screened, seven positive clones were isolated and purified. After extraction and purification, the DNA was analyzed by restriction enzymes: One clone (A1) has a 3.5 kb BamHI fragment and a 3 kb PstI fragment; another clone (B1) has a 9.4 kb BamHI fragment and a 9.4 kb PstI 30 fragment; and 5 clones have a 9.4 kb BamHI fragment and a 10 kb PstI fragment. These results are similar to those obtained by Southern analysis of P. gingivalis DNA and are consistent with the existence of at least two Arg-gingipain genes. The A1 clone was chosen for sequencing because the expected DNA size to encode 35 a 50-kDa protein is approximately 1.35 kb. The 3.159 kb PstI/BamHI fragment from clone A1 was subsequently subcloned into pBluescript SK(-) as a PstI fragment and a SmaI/BamHI fragment.

and into M13mp18 and 19 as a *PstI* fragment and a *PstI/BamHI* fragment and sequenced. In order to clone the stop codon of gingipain-1, which was missing in the *PstI/BamHI* fragment, *PstI/HindIII* double digested *P. gingivalis* DNA clones were 5 hybridized with ^{32}P -labeled GIN-14-20 (SEQ ID NO:9) (nucleotides 2911-2930 of SEQ ID NO:3) localized at the 3' end of this clone. A *PstI/HindIII* fragment of approximately 4.3 kb was identified and cloned into pbluescript SK(-). Smaller fragment (*PstI/Asp713* and *BamHI/HindIII*) was also subcloned into M13mp18 and 19.

10

SEQ ID NO:3 is the DNA sequence of the 3159 bp *PstI/BamHI* fragment (see Table 1).

TABLE 1
Nucleotide sequence and deduced
amino acid sequence of an Arg-gingipain

10	20	30	40	
CTG CAG ACG GCT GGT AAA GAC CGC CTC CGG ATC GAG GGC TTT GAG ACC				
GAC GTC TCC CGA CCA TTT CTG GCG GAG CCC TAG CTC CGG AAA CTC TGC				
50	60	70	80	90
GGC ACA AGC CGC CGC AGC CTC CTC TTC GAA CGT GTC TCG AAC GTC CAC				
CGG TGT TCG GCG GCG TCG GAG GAG CTT CCA CAG AGC TTG CAG GTG				
100	110	120	130	140
ATC GGT GAA TCC GCA GTG CTC ATT GGC ATT GAG CAG CAC CGA CCT				
TAG CCA CCT AGG CAT CGT CAC GAG TAA CGG TAA CTC GTC GTG CCT CCA				
150	160	170	180	190
GTC CGG CAT CAG ATA TAT TTT CAT CAG TCG ATT ATT AGC GTC TCG GTC				
CAC CGC GTC GTC TAT ATA AAA GCA GTC ACC TAA TAA TCC CAT AGC CGG				
200	210	220	230	240
AGA AAA AGC CCT CCG AAT CCG ACA AAG ATA GTC GAA AGA GAG TGC ATC				
TCT TTT TCG GAA CGC TTA CGC TGT TTC TAT CAT CCT TCT CTC ACG TAC				
250	260	270	280	
TGA AAA CAG ATC ATT CGA CGA TAA TCG ATC AAC TGA AAA CGC AGG AGT				
ACT TTT GTC TAG TAA CCT CCT AAT AGC TAG TTG ACT TTT CGG TCC TCA				
290	300	310	320	330
TGT TTT CGG TTT TGG TTC GGA AAA TTA CCT GAT CAG CAT TTG TAA AAA				
ACA AAA CGC AAA ACC AAG CCT TTT AAT GCA CTC CTC GTC AGC ATT TTT				
340	350	360	370	380
CCT CGC CGG AGA ATT TTT TCG TTT TGG CGC GAG AAT TAA AAA TTT TTC				
GCA CGG CGC TTT TAA AAA ACC AAA ACC CGG CTC TTA ATT TTT AAA AAC				
390	400	410	420	430
GAA CCA CAG CGA AAA AAA TCT CGC GCG GTT TTC TCA GGA TTT ACA GAC				
CCT GGT GTC CCT TTT TTT AGA GCG CGG CGA AAG AGT CCT AAA TGT CTG				
440	450	460	470	480
CAC AAA CGG AGC ATT TTC GGT TCG TAA TTC ATC GAA GAG ACA GGT TTT				
GTC TTA CGC TCG TAA AAG CGA AGC ATT AAG TAG CCT CTC TGT CCA AAA				
490	500	510	520	
ACC CGA TTG AAA TCA GAG AGA GAA TAT CGG TAC TCC AGC GGT TCA TCC				
TGG CCT AAC TTT AGT CTC TCT CCT ATA CGC ATT AGC TTG CGA AGT AGG				
530	540	550	560	570
TAA TAT CAG AGG TAA AAA GAT ATG GCA CGC TCA TCG AGG AGC TCA TTG				
AAT ATA GTC TCC AAT TTT CTC TAC CAT CGG AGT AGC TCC TCG ACT AAC				
580	590	600	610	620
GCT TAG TAG CGC AGA CCT TCT TAA GAG ACT ATC CGC AGC TAC AGG AAC				
CGA ATC ATC CAC CCT GCA AGA ATT CTC TCA TAG CGG TGG ATG TCC TTC				
630	640	650	660	670
TTC ATG CGA CGC MAG CGA AAC GAG CGA AGC TTC CGA GAC CGG ACT GAT				
AAC TAC CGT CGG TTC CCT TTC CTC CCT TAG AGG CCT CGG CGG TCA GTC				

TABLE 1 (cont'd)

21

680	690	700	710	720
ATC AAA AGG ATG AAA CGA CTT TTC CAT ACG ACA ACC AAA TAG CGG TCT TAG TTT TCC TAC TTT GCT GAA AAG GTA TGC TGT TGG TTT ATC CCC ACG				
730	740	750	760	
ACG GTA GAC GAA TGC AAA CCC AAT ATG AGG CGA TCA ATC AAT CCG AAT TGC CAT CTG CTT ACC TTT CCC TTA TAC TCC CCT ACT TAG TTA CCC TTA				
770	780	790	800	810
GAC AGC TTT TGG GCA ATA TAT TAT GCA TAT TTT GAT TCG CGT TTA ARG CTG TCG AAA ACC CGT TAT ATA ATA CGT ATA AAA CTA AGC GCA AAT TTC				
820	830	840	850	860
GAA AAG TGC ATA TAT TTG CGA TTG TGG TAT TTC TTT CGG TTT CTA TGT CTT TTC ACG TAT ATA AAC GCT AAC ACC ATA AAG AIA GCC AAA GAT ACG				
870	880	890	900	910
GAA TTT TGT CTC CGA AGA AGA CTT TAT AAT GCA TTA ATA CGG AAG GGG CTT AAA ACA GAG GGT TGT TCT GAA ATA TTA CCT ATT TAT CTC TTC CCC				
920	930	940	950	960
TAC TAC ACA GTA AAA TCA TAT TCT AAT TTC ATC AAA ATG AAA AAC TTG ATG ATG CCT TGT TTT AGT ATA CGA TTA AAG TAG TTT TAC TTT TTG AAC M K N L				
970	980	990	1000	
AAC AAC TTT CGT TCG ATT CCT CTT TGC TCT TCC TTA TTA GGA CGA ACG TTC TTC AAA CAA AGC TAA CGA GAA ACG AGA AGG AAT AAT CCT CCT TAC N K F V S I A L C S S L L G G H				
1010	1020	1030	1040	1050
GCA TTT CGG CGG ACG ACA GAG TTG CGA CGC AAT CGG AAT GTC AGA TTG CGT AAA CGC GTC GTC TCT AAC CCT CGG TTA CGC TTA CGG TCT AAC A F A Q Q T E L G R N ? N V R L				
1060	1070	1080	1090	1100
CTC GAA TCC ACT CGC CAA TCG GTG ACA AAG GTT CGG TTC CGT ATG GAC GAG CTT ACG TGA CTC CTT ACC CGC TCT TTC CAA CTC AAG CGA TAC CTC L E S T Q S V T R V Q F R M D				
1110	1120	1130	1140	1150
AAC CTC AAG TTC ACC GAA GTT CAA ACC CCT AAG CGA ATC GGA CAA CGT TTG GAG TTC AAG TCG CCT CAA GTT CGG CGA TTC CCT TAG CCT GTT CGC N L K ? T E V Q T P K G I G Q V				
1160	1170	1180	1190	1200
CCG ACC TAT ACA GAA CGG GTT AAT CCT TCC CAA AAA CGG ATG CCT ACG GCC TCG ATA TGT CCT CCC CAA TCA GAA ACG CCT TTT CGG TAC CGA TGC ? T Y T E G V N L S E K G M ? T				
1210	1220	1230	1240	
CTT CGC ATT CTA TCA CGC TCT TTG GCG GTT TCA GAC ACT CCT CGG ATG GAA CGG TAA GAT AGT CGG AGA AAC CGC CAA ACT CCT TCA CGA CTC TAC L ? I L S R S L A V S D T R E K				
1250	1260	1270	1280	1290
ACG GTA GAG GTT GTT TCC TCA AAG TTC ATC CGA AAG AAA AAT GTC CTG TTC CAT CCT CAA CAA AGG AGT TTC AAG TAC CGT CCT TCC TTT TTA CGC GAC K V E V V S S K F I E X X N V L				

TABLE 1 (cont'd)

22

1300	1310	1320	1330	1340
ATT GCA CCC TCC AAG GGC ATG ATG CCT AAC GAA GAT CCG AAA AAG TAA CCT CGG AGG TTC CGG TAC TAA TAC GCA TGG CCT CTR CGG TTT TTC I A P S R G M I X R N E D P K X				
1350	1360	1370	1380	1390
ATC CCT TAC GTT TAT CGA AAG AGC TAC TCG CAA AAC AAA TTC TTC CGG TAG CGA ATG CAA ATA CCT TTC TCG ATG AGC GTT TTC TTT AAG AAG CGC I ? Y V Y G K S Y S Q N K F ? ?				
1400	1410	1420	1430	1440
GGA GAG ATC GCC ACC CTT GAT GAT CCT TTT ATC CTT CGT GAT GTG CGT CCT CTC TAG CGG TGC GAA CTA CTR CGA AAA TAG GAA GCA CTA CAC CGA G E I A T L D D P F I L R D V R				
1450	1460	1470	1480	
GGA CAG CTT GTA AAC TTT GCG CCT TTG CAG TAT AAC CCT GTG ACA AAG CCT GTC CAA CAT TTC AAA CGC CGA AAC GTC ATA TTG CGA CAC CGT TTC G Q V V N F A P L Q Y N P V T K				
1490	1500	1510	1520	1530
ACG TTG CGC RTC TAT ACC GAA ATC ACT GTG CGA GTG ACC GAA ACT TCG TGC AAC CGG TAG ATA TGC CTT TAG TGA CAC CGT CAC TCG CCT TGA AGC T L R I Y T E I T V A V S E T S				
1540	1550	1560	1570	1580
GAA CAA CGC AAA AAT ATC CTG AAC AAG AAA CCT ACA TTT CCC CGC TTT CTT GTT CGG TTT TTA TAA GAC TTS TTC TTT CGA TGT AAA CGG CGG AAA E Q G K N I L N R K G T F A G F				
1590	1600	1610	1620	1630
GAA GAC ACA TAC AAG CGC ATG TTC ATG AAC TAC GAG CGG CGG CGT TAC CTT CTG TGT ATG TTC CGG TAC AAG TAC TTG ATG CTC CGC CCC CGA ATG E D T Y R R M F N N Y E P G R Y				
1640	1650	1660	1670	1680
ACA CGG GTA GAG GAA AAA CGA AAT GGT CGT ATG ATC GTC ATC GTA CGC TGT CGC CAT CTC CTT TTT GTT TTA CGA GCA TAC TAC CAG TAC CAT CGG I P V E S K O N G R M I V I V A				
1690	1700	1710	1720	
AAA AAG TAT GAC CGA GAT ATT AAA GAT TTT GGT GAT TGG AAA AAC CGA TTT TTC ATA CTC CCT CTR TAA TTT CTR AAC CGA CTR AAC TTT TGG CTT K K V I G D I K D F V D W R N Q				
1730	1740	1750	1760	1770
CCC GGT CTC CCT ACC GAG GTG AAA CTC CGA GAA GAT ATT GCT TCT CGG CGG CGA GAG CGA TGG CTC CGC TTT CGC CCT CTT CGA TAA CGA AGA CGG R C I R T E V R V A E D I A S ?				
1780	1790	1800	1810	1820
GTT ACA CCT AAT CCT ATT CAG CAG TTC GTT AAG CGA CGA TAC GAG AAA CAA TGT CGA TTA CGA TAA GTC CGC AAG CGA TTC GTT CCT ATG CTC TTT V T A N A I Q Q F V K Q E Y E K				
1830	1840	1850	1860	1870
GAA CGT AAT GAT TTG ACC TAT GGT CCT TTG GGT CGG GAT CAC AAA CGT CTT CGA TTA CTR AAC TGC ATA CGA CGA AAC CAA CGG CGA GTG TTT CTA E G N D L T Y V I L V C D H X K D				

TABLE 1 (cont'd)

1880	1890	1900	1910	1920
ATT CCT GCC AAA ATT ACT CCC GGG ATC AAA TCC GAC CAG GTA TAT GGA TAA CGA CGG TTT TAA TGA GGC CCC ATC TTC AGG CTG CTC CAT ATA CCT I P A R I T ? G I X S D Q V Y C				
1930	1940	1950	1960	
CAA ATA GTA GGT ATT GAC CAC TAC AAC GAA GTC TTC ATC GGT CGT TTC GTT TAT CAT CCA TTA CTG CTC ATC TTC CTT CAC AAC TAG CCA CGA AAC Q I V C N D H Y N E V F I G R F				
1970	1980	1990	2000	2010
TCA TGT GAC ACC AAA GAG GAT CTG AAG ACA CAA ATC GAT CGG ACT ATT AGT ACA CTC TCG TTT CTC CTA GAC TTC TGT GTT TAG CTA GCC TGA TAA S C E S K E D L K T Q I D R F I				
2020	2030	2040	2050	2060
CAC TAT GAG CGC ATT ATA ACC ACC GAA GAC AAA TGG CTG CGT CAG GGT CTG ATA CTC CGG TAA TAT TGG TGC CTT CTG TTT ACC GAG CCA GTC CGA H Y E R N I T T E D K W L G Q A				
2070	2080	2090	2100	2110
CTT TGT ATT GCT TCG GCT GAA GGA CGC CCA TCC GCA GAC ATT GGT GAA GAA ACA TAA CGA AGC CGA CTT CCT CGG CGT AGG CGT CTG TTA CGA CCT CTT L C I A S A E G G P S A D N G E				
2120	2130	2140	2150	2160
ACT GAT ATC CGG CAT GAG ATT GTA ATC GGC ATT CTG CTT ACC CAG TAT TCA CTA TAG GTC GTC TAA CAT TAG CGG TTA GAC GAA TGG GTC ATA S D I Q H E N V I A N L D T Q ?				
2170	2180	2190	2200	
GGC TAT ACC AAG ATT ATC AAA TGT TAT GAT CCC CGA GTC ACT CCT AAA CCC ATA TGG TTC TAA TAG TTT ACA ATA CTA CGC CCT CAT TGA CGA TTT G Y T K I I X C Y D P G V T P K				
2210	2220	2230	2240	2250
AAC ATT ATT GAT GCT TTC AAC CGA GGA ATC TCC TTG CTC AAC TAT ACC TTG TAA TAA CTA CGA AAC TTG CCT CCT TRG AGC AAC CAG TTG ATA TGC N I I D A F N G G I S L V N Y T				
2260	2270	2280	2290	2300
GCC CAC CGT ACC GAA ACA GCT TGG CGT ACC TCT CAC TTC CGC ACC ACT CGG CTG CGA TCG CTT TGT CGA ACC CGA TGC AGA GTC AAC CGG TGG TGA G H C S E T A W G T S H F G T T				
2310	2320	2330	2340	2350
CAT GTC AAG CGG CTT ACC AAC AGC AAC CGG CTA CGG TTT ATT TTC GAC GTA CAC TCC GTC CGA TGG TGC TGC CTC CAT CGC AAA TAA AAC CGG H V K Q L T N S N Q L P F I F D				
2360	2370	2380	2390	2400
GTA CCT TGT CTG AAT GGC GAT TTC CGA TTC AGC ATG CCT CGC TTC GCA CAT CGA ACA CAC TTA CGG CTA AAC GAT AAC TGC TAC CGA AGC AAC CCT V A C V N G D F I F S M ? C F A				
2410	2420	2430	2440	
CAA CGC CTG ATG CGT CGA CAA AAA GAT CGT AAC CGG ACA CCT ACT GTT CTT CGG CGC TAC CGA CGT CCT TTT CTA CGA TTC CGC TGT CGA TGA CGA E A L M R A Q K D G K F T G T V				

TABLE 1 (cont'd)

24

2450	2460	2470	2480	2490
GCT ATC ATA CGG TCT ACG ATC AAC CAG TCT TGG GCT TCT CCT ATG CGG CGA TAC TAT CGG AGA TCC TAG TTG GTC AGA ACC CGA AGA CGA TAC CGG A I A S T I N Q S W A S ? M R				
2500	2510	2520	2530	2540
CGG CAC GAT GAG ATG AAC GAA ATT CTG TGC GAA AAA CAC CGG AAC AAC CCC GTC CCA CGG TAC TTG CCT TAA GAC ACG CCT TCT GTG CGG TGG TTG G Q D E M N E I L C E K H ? N N				
2550	2560	2570	2580	2590
ATC AAC CCT ACT TTC GGT GGT GTC ACC ATG AAC GGT ATG TTT CCT ATG TAG TTC GCA TGA AAC CCA CCA CAG TGG TAC TTG CCA TAC AAA CGA TAC I K R T F G G V T M N G M F A M				
2600	2610	2620	2630	2640
CTG GAA AAG TAT AAA AAG GAT GGT GAG AAG ATG CTC GAC ACA TGG ACT CAC CCTT TTC ATA TTT TTC CCA CCA CTC TTC TAC GAG CTG TGT ACC TCA V E R Y X D G E R M L D T W T				
2650	2660	2670	2680	
GTT TTC GGC GAC CCC TCC CTG CTC GTT CGT ACA CCT GTC CGG ACC AAA CAA AAG CCC CTG GGG AGC GAC GAC CAA GCA TGT GAA CAG CGG TGG TTT V F G D P S L L V R T L V P T X				
2690	2700	2710	2720	2730
ATG CAG CTT ACG CCT CGT CAG ATT AAT TTG ACG GAT GCT TCA GTC TAC GTC CAA TGC CGA CGG CGA GTC TTA AAC TGG CCA CGA AGT CAG M Q V T X P A Q I N E T D A S V				
2740	2750	2760	2770	2780
AAC GTA TCT TGC GAT TAT AAT GGT GCT ATT GCT ATT ACC ATT TCA GCC AAT TTC CAT AGA ACG CCA ATA TTA CCA CGA TAA CGA TGG TAA ACT CGG TTT N V S C D Y N G I A T S A N				
2790	2800	2810	2820	2830
GGA AAG ATG TTC GGT TCT GCA GTT GTC GAA AAT CGA ACA CCT ACA ATT CCT TTC TAC AAG CCA AGA CCT CAA CAG CCT TTA CCT TGT CGA TGT TAG G K M F G S A V V E N G T A T I				
2840	2850	2860	2870	2880
AAT CTG ACA CCT CTG ACG ATT GAA ACC ACG CCT ACC CCT ACA GTC GTC TTA GAC TGT CCA GAC TGT TTA CCT TGG TCC GAA TGG GAA TGT CAT CAA N I T G L T N E S T L T L T V V				
2890	2900	2910	2920	
GGT TAC AAC AAA GAG ACG CCT ATT AAG ACC ATC AAC ACT AAT GGT GAG CCA ATG TTG TTT CTC TCC CCA TTA TTC TGG TAG TTG TCA TTA CGA CTC G Y N K S T V I K T I N T N G E				
2930	2940	2950	2960	2970
CCT AAC CCC TAC CGG CCC GTT TCC AAC TTG ACA CCT ACA ACG CGG GGT CCA TTG GGG ATG CTC CGG CGA AAC TTG AAC TGT CGA TGT TCC CCT CCA P N P Y Q P V S N L T A T T Q G				
2980	2990	3000	3010	3020
CAC AAA GTC ACG CCT ACG TGG GAT CGA CGG AAC ACG AAA ACC AAC ATT CGA CCC CCT TGC GAG TTG ACC CCT CGT GGC TGG TCC TTT TGG TTA CGT Q R V T I K W D A ? S T X T N A >				

TABLE 1 (cont'd)

25

3030	3040	3050	3060	3070
ACC ACT ATT ACC GCT CCC AGC GTG GAT GGC ATG CCA GAA TTG GTT CTT	TGG TGA TTA TGG CGA GCG TCG CAC CTA CGG TAC GCT CCT AAC CGA GAA	T T N T A ? S V D G I R E L V I		
3080	3090	3100	3110	3120
CTG TCA GTC ACC GAT GCG CCC GAA CCT CCT CGG AGG GGT CGG GCG CGG	GAC ACT CGG TCG CTA CGG CGG CCT GAA CGA CGG CCT CGA GTC CGG CCT	I S V S D A ? S I L R S G Q A I		
3130	3140	3150		
ATT CCT CCT GAA CCT CAC GAT GTT CGG ATT GAT GCA TCC	TAA CCA GAA CCT CGA GTG CCA CGA ACC TTA CCA CCT AGG	I V L E I H D V W N D G S >		

Exemplified nucleotide sequences encoding a mature Arg-gingipain, termed an Arg-gingipain-2 herein, extends from 1630-3105 in SEQ ID NO:3 and in SEQ ID NO:10. The first ATG appears at nucleotide 949 and is followed by a long open reading frame (ORF), of 5111 bp in Table 2 (SEQ ID NO:10). This ORF was the largest one observed. However, the first ATG is following by 8 others in frame (at nucleotides 1006, 1099, 1192, 1246, 1315, 1321, 1603, and 1609). The most likely candidate to initiate translation is currently unknown. Which of these initiation codons are used in translation of the Arg-gingipain-2 precursor can be determined by expression of the polyprotein in bacteria and subsequent amino-terminal sequence analysis of proprotein intermediates. The sequence derived from 5' noncoding sequences is composed of 948 bp. The primary structure of the mature Arg-gingipain molecule can be inferred from the empirical amino-terminal and carboxy-terminal sequences and molecular mass. Thus, mature Arg-gingipain-2 has an amino terminus starting at nucleotide residue 1630 in SEQ ID NO:3 and at amino acid 1 in SEQ ID NO:4. As expected for an arginine-specific protease, the mature protein is cleaved after an arginine residue. The 50 kDa and the 44 kDa bands from Bz-L-Arg-pNa activity peaks have an identical sequence to that deduced amino acid sequence of gingipain, encoded respectively at nucleotides 1630-1695 and at nucleotides 3106-3156. From these data, the carboxyl terminus is most likely derived from autoproteolytic processing after the arginine residue encoded at 3103-3105 where the amino terminus encoding sequence of a hemagglutinin component starts (nucleotide 3106). The deduced 492 amino acids of gingipain-2 give rise to a protease molecule with a calculated molecular weight of 54 kDa which correlates well with the molecular mass of 50 kDa determined by SDS-PAGE analysis. Tables 1 and 2 (see also SEQ ID NO:10 and 11) presents the coding sequence and deduced amino acid sequence of gingipain-2. The first nucleotide presented in the sequence belongs to the *Pst*I cloning site and is referred as nucleotide 1. Bold face letters indicate the potential sites of initiation ATG and the first codon of the mature gingipain-2. The amino terminal sequence of gingipain-2 and the amino terminal

sequence of 44 kDa bands from Bz-L-Arg-pNa activity peaks are underlined.

5 Table 2 (corresponding to SEQ ID NOS:10-11) presents the nucleotide sequence encoding the complete preproprotein sequence, including both the protease component and the hemagglutin component(s) of HMW Arg-gingipain. The coding sequence extends from an ATG at nucleotide 949 through a TAG stop codon at nucleotide 6063 in SEQ ID NO:10. The deduced amino acid
10 sequence is given in SEQ ID NO:11.

TABLE 2

28

Sequence Range: 1 to 7266

>Pst1
 CTGCAGAGGG CTGGTAAAGA CGGCCTCGGG ATCGAGGCCT TTGAGACGGG CACAAGCCGC CGCAGCCCTC
 100
 TCTTCGAAGG TGTCTCGAAC GTCCACATCG GTGAATCCGT AGCAGTGCTC ATTGCCATTG AGCAGCACCG
 200
 AGGTGTGGCG CATCAGATAT ATTTTCATCA GTGGATTATT AGGGTATCGG TCAGAAAAAG CCTTCCGAAT
 >Cla1
 CCGACAAAAGA TAGTAGAAAG AGAGTGCATC TGAAAACAGA TCATTCGAGG ATTATCGATC AACTGAAAAG
 300
 GCAGGAGTTG TTTTGCCTT TGTTTCGGAA AATTACCTGA TCAGCATTG TAAAAACGTG GCGCGAGAAT
 400
 TTTTCGTT TGGCGCGAGA ATTAAAATT TTTGGAACCA CAGCGAAAAA AATCTCGGC CGTTTCTCA
 GGATTTACAG ACCACAATCC GAGCATTTC GGTTCGTAAT TCATCGAAGA GACAGGTTT ACCGCATTGA
 500
 AATCAGAGAG AGAATATCCG TAGTCCAACG GTTCATCCTT ATATCAGAGG TTAAAAGATA TGGTACGCTC
 600
 ATCGAGGAGC TGATTGGCTT AGTAGGTGAG ACTTTCTAA GAGACTATCG GCACCTACAG GAAGTTCATG
 700
 GCACACAAAGG CAAAGGAGGC AATCTCGCA GACCGGACTC ATATCAAAG GATGAAACGA CTTTCCATA
 CGACAACCAA ATAGCCGTCT ACGGTAGACG AATGCAAACC CAATATGAGG CCATCAATCA ATCCGAATGA
 800
 CAGCTTTGG GCAATATATT ATGCATATTT TGATTGGCGT TTAAAGGAAA AGTGCATATA TTTGGATTG
 900
 TGGTATTCTT TTCCGGTTCT ATGTGAATT TGTCTCCCAA GAAGACTTTA TAATGCATAA ATACAGAAGG
 GGTACTACAC AGTAAAATCA TATTCTAATT TCATCAAATG AAA AAC TTG AAC AAG TTT GTT TCG
 M K N L N K F V S>
 1000
 ATT GCT CTT TGC TCT TCC TTA TTA GGA GGA ATG GCA TTT GCG CAG CAG ACA GAG TTG
 I A L C S S L L G G M A F A Q Q T E L>
 GGA CGC AAT CCG AAT GTC AGA TTG CTC GAA TCC ACT CAG CAA TCG GTG ACA AAG GTT
 G R N P N V R L L E S T Q Q S V T K V>
 1100
 TAG TTC CGT ATG GAC AAC CTC AAG TTC ACC GAA CAA ACC CCT AAG GGA ATC GGA
 Q F R M D N L K F T E V Q T P K G I G>

RECTIFIED SHEET (RULE 91)

Table 2 (contd.)

29

CAA GTG CCG ACC TAT ACA GAA GGG GTT AAT CTT TCC GAA AAA GGG ATG CCT ACG CTT
 Q V P T Y T E G V N L S E K G M P T L>

 CCC ATT CTA TCA CGC TCT TTG GCG GTT TCA GAC ACT CGT GAG ATG AAG GTA GAG GTT
 P I L S R S L A V S D T R E M K V E V>
 1300

 GTT TCC TCA AAG TTC ATC GAA AAG AAA AAT GTC CTG ATT GCA CCC TCC AAG GGC ATG
 V S S K F I E K K N V L I A P S K G M>

 ATT ATG CGT AAC GAA GAT CCG AAA AAG ATC CCT TAC GTT TAT GGA AAG AGC TAC TCG
 I M R N E D P K K I P Y V Y G K S Y S>
 1400

 CAA AAC AAA TTC TTC CCG GGA GAG ATC GCC ACG CTT GAT GAT CCT TTT ATC CTT CGT
 Q N K F F P G E I A T L D D P F I L R>

 GAT GTG CGT GGA CAG GTT GTA AAC TTT GCG CCT TTG CAG TAT AAC CCT GTG ACA AAG
 D V R G Q V V N F A P L Q Y N P V T K>
 1500

 ACG TTG CGC ATC TAT ACG GAA ATC ACT GTG GCA GTG AGC GAA ACT TCG GAA CAA GGC
 T L R I Y T E I T V A V S E T S E Q G>
 1600

 AAA AAT ATT CTG AAC AAG AAA GGT ACA TTT GCC GGC TTT GAA GAC ACA TAC AAG CGC
 K N I L N K K G T F A G F E D T Y K R>

 ATG TTC ATG AAC TAC GAG CCG GGG CGT TAC ACA CCG GTA GAG GAA AAA CAA AAT GGT
 M F M N Y E P G R Y T P V E E K Q N G>
 1700

 CGT ATG ATC GTC ATC GTA GCC AAA AAG TAT GAG GGA GAT ATT AAA GAT TTC GTT GAT
 R M I V I V A K K Y E G D I K D F V D>

 TGG AAA AAC CAA CGC GGT CTC CGT ACC GAG GTG AAA GTG GCA GAA GAT ATT GCT TCT
 W K N Q R G L R T E V K V A E D I A S>
 1800

 CCC GTT ACA GCT AAT GCT ATT CAG CAG TTC GTT AAG CAA GAA TAC GAG AAA GAA GGT
 P V T A N A I Q Q F V K Q E Y E K E G>

 AAT GAT TTG ACC TAT GTT CTT TTG GTT GGC GAT CAC AAA GAT ATT CCT GCC AAA ATT
 N D L T Y V L L V G D H K D I P A K I>
 1900

 ACT CCG GGG ATC AAA TCC GAC CAG GTA TAT GGA CAA ATA GTA GGT AAT GAC CAC TAC
 T P G I K S D Q V Y G Q I V G N D H Y>
 2000

 AAC GAA GTC TTC ATC GGT CGT TTC TCA TGT GAG AGC AAA GAG GAT CTG AAG ACA CAA
 N E V F I G R F S C E S K E D L K T Q>

 >Clal

 ATC GAT CGG ACT ATT CAC TAT GAG CGC AAT ATA ACC AC G GAA GAC AAA TGG CTC GGT
 I D R T I H Y E R N I T T E D K W L G>

RECTIFIED SHEET (RULE 91)

Table 2 (contd.)

30

2100

CAG GCT CTT TGT ATT GCT TCG GCT GAA GGA GGC CCA TCC GCA GAC AAT GGT GAA AGT
 Q A L C I A S A E G G P S A D N G E S>

>EcoR5

GAT ATC CAG CAT GAG AAT GTA ATC GCC AAT CTG CTT ACC CAG TAT GGC TAT ACC AAG
 D I Q H E N V I A N L L T Q Y G Y T K>

2200

ATT ATC AAA TGT TAT GAT CCG GGA GTA ACT CCT AAA AAC ATT ATT GAT GCT TTC AAC
 I I K C Y D P G V T P K N I I D A F N>

GGA GGA ATC TCG TTG GTC AAC TAT ACG GGC CAC GGT AGC GAA ACA GCT TGG GGT ACG
 G G I S L V N Y T G H G S E T A W G T>

2300

TCT CAC TTC GGC ACC ACT CAT GTG AAG CAG CTT ACC AAC AGC AAC CAG CTA CCG TTT
 S H F G T T H V K Q L T N S N Q L P F>

>Sph1

2400

ATT TTC GAC GTA GCT TGT GTG AAT GGC GAT TTC CTA TTC AGC ATG CCT TGC TTC GCA
 I F D V A C V N G D F L F S M P C F A>

GAA GCC CTG ATG CGT GCA CAA AAA GAT GGT AAG CCG ACA GGT ACT GTT GCT ATC ATA
 E A L M R A Q K D G K P T G T V A I I>

2500

GCG TCT ACG ATC AAC CAG TCT TGG GCT TCT CCT ATG CGC GGG CAG GAT GAG ATG AAC
 A S T I N Q S W A S P M R G Q D E M N>

GAA ATT CTG TGC GAA AAA CAC CCG AAC AAC ATC AAG CGT ACT TTC GGT GTC ACC
 E I L C E K H P N N I K R T F G G V T>

2600

ATG AAC GGT ATG TTT GCT ATG GTG GAA AAG TAT AAA AAG GAT GGT GAG AAG ATG CTC
 M N G M F A M V E K Y K K D G E K M L>

GAC ACA TGG ACT GTT TTC GGC GAC CCC TCG CTG CTC GTT CGT ACA CTT GTC CCG ACC
 D T W T V F G D P S L L V R T L V P T>

2700

AAA ATG CAG GTT ACG GCT CCG GCT CAG ATT AAT TTG ACG GAT GCT TCA GTC AAC GTA
 K M Q V T A P A Q I N L T D A S V N V>

TCT TGC GAT TAT AAT GGT GCT ATT GCT ACC ATT TCA GCC AAT GGA AAG ATG TTC GGT
 S C D Y N G A I A T I S A N G K M F G>

>Pst1

2800

TCT GCA GTT GTC GAA AAT GGA ACA GCT ACA ATC AAT CTG ACA GGT CTG ACA AAT GAA
 S A V V E N G T A T I N L T G L T N E>

2900

RECTIFIED SHEET (RULE 91)

Table 2 (contd.)

31

AGC ACG CTT ACC CTT ACA GTA GTT GGT TAC AAC AAA GAG ACG GTT ATT AAG ACC ATC
 S T L T L T V V G Y N K E T V I K T I>

AAC ACT AAT GGT GAG CCT AAC CCC TAC CAG CCC GTT TCC AAC TTG ACA GCT ACA ACG
 N T N G E P N P Y Q P V S N L T A T T>

3000

CAG GGT CAG AAA GTA ACG CTC AAG TGG GAT GCA CCG ACG ACG AAA ACC AAT GCA ACC
 Q G Q K V T L K W D A P S T K T N A T>

ACT AAT ACC GCT CGC ACG GTG GAT GGC ATA CGA GAA TTG GTT CTT CTG TCA GTC AGC
 T N T A R S V D G I R E L V L L S V S>

3100

GAT GCC CCC GAA CTT CTT CGC ACG GGT CAG GCC GAG ATT GTT CTT GAA GCT CAC GAT
 D A P E L L R S G Q A E I V L E A H D>

>BamH1

GTT TGG AAT GAT GGA TCC GGT TAT CAG ATT CTT TTG GAT GCA GAC CAT GAT CAA TAT
 V W N D G S G Y Q I L L D A D H D Q Y>

3200

GGA CAG GTT ATA CCC AGT GAT ACC CAT ACT CTT TGG CCG AAC TGT AGT GTC CCG GCC
 G Q V I P S D T H T L W P N C S V P A>

3300

AAT CTG TTC GCT CCG TTC GAA TAT ACT GTT CCG GAA AAT GCA GAT CCT TCT TGT TCC
 N L F A P F E Y T V P E N A D P S C S>

CCT ACC AAT ATG ATA ATG GAT GGT ACT GCA TCC GTT AAT ATA CCG GCC GGA ACT TAT
 P T N M I M D G T A S V N I P A G T Y>

3400

GAC TTT GCA ATT GCT GCT CCT CAA GCA AAT GCA AAG ATT TGG ATT GCC GGA CAA GGA
 D F A I A A P Q A N A K I W I A G Q G>

CCG ACG AAA GAA GAT GAT TAT GTA TTT GAA GCC GGT AAA AAA TAC CAT TTC CTT ATG
 P T K E D D Y V F E A G K K Y H F L M>

3500

AAG AAG ATG GGT AGC GGT GAT GGA ACT GAA TTG ACT ATA AGC GAA GGT GGT GGA AGC
 K K M G S G D G T E L T I S E G G G S>

GAT TAC ACC TAT ACT GTC TAT CGT GAC GGC ACG AAG ATC AAG GAA GGT CTG ACG GCT
 D Y T Y T V Y R D G T K I K E G L T A>

3600

ACG ACA TTC GAA GAA GAC GGT GTA GCT ACG GGC AAT CAT GAG TAT TGC GTG GAA GTT
 T T F E E D G V A T G N H E Y C V E V>

>BamH1

3700

AAG TAC ACA GCC CCC GTA TCT CCG AAG GTA TGT AAA GAC GTT ACG GTA GAA GGA TCC
 K Y T A G V S P K V C K D V T V E G S>

AAT GAA TTT GCT CCT GTA CAG AAC CTG ACC GGT AGT GCA GTC GGC CAG AAA GTA ACG

Table 2 (contd.)

32

N	E	F	A	P	V	Q	N	L	T	G	S	A	V	G	Q	K	V	T>	
>Asp718																			
3800																			
CTC	AAG	TGG	GAT	GCA	CCT	AAT	GGT	ACC	CCG	AAT	CCA	AAT	CCG	AAT	CCG	AAT	CCG	AAT	
L	K	W	D	A	P	N	G	T	P	N	P	N	P	N	P	N	P	N>	
CCC	GGA	ACA	ACA	ACA	ACA	CTT	TCC	GAA	TCA	TTC	GAA	AAT	GGT	ATT	CCT	GCC	TCA	TGG	AAG
P	G	T	T	T	L	S	E	S	F	E	N	G	I	P	A	S	W	K>	
>Cla1																			
3900																			
ACG	ATC	GAT	GCA	GAC	GGT	GAC	GGG	CAT	GGC	TGG	AAG	CCT	GGA	AAT	GCT	CCC	GGA	ATC	
T	I	D	A	D	G	D	G	H	G	W	K	P	G	N	A	P	G	I>	
GCT	GGC	TAC	AAT	AGC	AAT	GGT	TGT	GTA	TAT	TCA	GAG	TCA	TTC	GGT	CTT	GGT	GGT	ATA	
A	G	Y	N	S	N	G	C	V	Y	S	E	S	F	G	L	G	G	I>	
4000																			
GGA	GTT	CTT	ACC	CCT	GAC	AAC	TAT	CTG	ATA	ACA	CCG	GCA	TTG	GAT	TTG	CCT	AAC	GGA	
G	V	L	T	P	D	N	Y	L	I	T	P	A	L	D	L	P	N	G>	
4100																			
GGT	AAG	TTG	ACT	TTC	TGG	GTA	TGC	GCA	CAG	GAT	GCT	AAT	TAT	GCA	TCC	GAG	CAC	TAT	
G	K	L	T	F	W	V	C	A	Q	D	A	N	Y	A	S	E	H	Y>	
GCG	GTG	TAT	GCA	TCT	TCG	ACC	GGT	AAC	GAT	GCA	TCC	AAC	TTC	ACG	AAT	GCT	TTG	TTG	
A	V	Y	A	S	S	T	G	N	D	A	S	N	F	T	N	A	L	L>	
4200																			
GAA	GAG	ACG	ATT	ACG	GCA	AAA	GGT	GTT	CCG	TCG	CCG	GAA	GCT	ATT	CGT	GGT	CGT	ATA	
E	E	T	I	T	A	K	G	V	R	S	P	E	A	I	R	G	R	I>	
CAG	GGT	ACT	TGG	CGC	CAG	AAG	ACG	GTA	GAC	CTT	CCC	GCA	GGT	ACG	AAA	TAT	GTT	GCT	
Q	G	T	W	R	Q	K	T	V	D	L	P	A	G	T	K	Y	V	A>	
4300																			
TTC	CGT	CAC	TTC	CAA	AGC	ACG	GAT	ATG	TTC	TAC	ATC	GAC	CTT	GAT	GAG	GTT	GAG	ATC	
F	R	H	F	Q	S	T	D	M	F	Y	I	D	L	D	E	V	E	I>	
AAG	GCC	AAC	GGC	AAG	CGC	GCA	GAC	TTC	ACG	GAA	ACG	TTC	GAG	TCT	TCT	ACT	CAT	GGA	
K	A	N	G	K	R	A	D	F	T	E	T	F	E	S	S	T	H	G>	
>Cla1																			
4400																			
GAG	GCA	CCG	GGC	GAA	TGG	ACT	ACT	ATC	GAT	GCC	GAT	GGC	GAT	GGT	CAG	GGT	TGG	CTC	
E	A	P	A	E	W	T	T	I	D	A	D	G	D	G	Q	G	W	L>	
4500																			
TGT	CTG	TCT	TCC	GGA	CAA	TTG	GAC	TGG	CTG	ACA	GCT	CAT	GGC	GGC	ACC	AAC	GTA	GTA	
C	L	S	S	G	Q	L	D	W	L	A	H	G	G	T	N	V	V>		
GCC	TCT	TTC	TCA	TGG	AAT	GGA	ATG	GCT	TTG	AAT	CCT	GAT	AAC	TAT	CTC	ATC	TCA	AAG	
A	S	F	S	W	N	G	M	A	L	N	P	D	N	Y	L	I	S	K>	
4600																			

Table 2 (contd.)

33

GAT GTT ACA GGC GCA ACG AAG GTA AAG TAC TAC TAT GCA GTC AAC GAC GGT TTT CCC
D V T G A T K V K Y Y Y A V N D G F P>

GGG GAT CAC TAT GCG GTG ATG ATC TCC AAG ACG GGC ACG AAC GCC GGA GAC TTC ACG
G D H Y A V M I S K T G T N A G D F T>

4700

GTT GTT TTC GAA GAA ACG CCT AAC GGA ATA AAT AAG GGC GGA GCA AGA TTC GGT CTT
V V F E E T P N G I N K G G A R F G L>

TCC ACG GAA GCC AAT GGC GCC AAA CCT CAA AGT GTA TGG ATC GAG CGT ACG GTA GAT
S T E A N G A K P Q S V W I E R T V D>

4800

TTG CCT GCG GGC ACG AAG TAT GTT CCT TTC CGT CAC TAC AAT TGC TCG GAT TTG AAC
L P A G T K Y V A F R H Y N C S D L N>

>Nco1

4900

TAC ATT CCT TTG GAT GAT ATT CAG TTC ACC ATG GGT GGC AGC CCC ACC CCG ACC GAT
Y I L L D D I Q F T M G G S P T P T D>

TAT ACC TAC ACG GTG TAT CGT GAC GGT ACG AAG ATC AAG GAA GGT CTG ACC GAA ACG
Y T Y T V Y R D G T K I K E G L T E T>

5000

ACC TTC GAA GAA GAC GGC GTA GCT ACA GGC AAT CAT GAG TAT TGC GTG GAA GTG AAG
T F E E D G V A T G N H E Y C V E V K>

TAC ACA GCC GGC GTA TCT CCG AAA GAG TGC GTA AAC GTA ACT ATT AAT CCG ACT CAG
Y T A G V S P K E C V N V T I N P T Q>

5100

TTC AAT CCT GTA AAG AAC CTG AAG GCA CAA CCG GAT GGC GGC GAC GTG GTT CTC AAG
F N P V K N L K A Q P D G G D V V L K>

TGG GAA GGC CCG ACC GCA AAA AAG ACA GAA GGT TCT CGT GAA GTA AAA CGG ATC GGA
W E A P S A K K T E G S R E V K R I G>

5200

GAC GGT CTT TTC GTT ACG ATC GAA CCT GCA AAC GAT GTA CGT GCC AAC GAA GCC AAG
D G L F V T I E P A N D V R A N E A K>

5300

GTT GTG CTC GCA GCA GAC AAC GTA TGG GGA GAC AAT ACG GGT TAC CAG TTC TTG TTG
V V L A A D N V W G D N T G Y Q F L L>

GAT GCC GAT CAC AAT ACA TTC GGA AGT GTC ATT CCG GCA ACC GGT CCT CTC TTT ACC
D A D H N T F G S V I P A T G P L F T>

5400

GGA ACA GCT TCT TCC AAT CTT TAC AGT GCG AAC TTC GAG TAT TTG ATC CCG GCC AAT
G T A S S N L Y S A N F E Y L I P A N>

GCC GAT CCT GTT GTT ACT ACA CAG AAT ATT ATC GTT ACA GGA CAG GGT GAA GTT GTA
A D P V V T T Q N I I V T G Q G E V V>

Table 2 (contd.)

34

5500

ATC CCC GGT GGT GTT TAC GAC TAT TGC ATT ACG AAC CCG GAA CCT GCA TCC GGA AAG
 I P G G V Y D Y C I T N P E P A S G K>

ATG TGG ATC GCA GGA GAT GGA GGC AAC CAG CCT GCA CGT TAT GAC GAT TTC ACA TTC
 M W I A G D G G N Q P A R Y D D F T F>

5600

GAA GCA GGC AAG AAG TAC ACC TTC ACG ATG CGT CGC GCC GGA ATG GGA GAT GGA ACT
 E A G K K Y T F T M R R A G M G D G T>

5700

GAT ATG GAA GTC GAA GAC GAT TCA CCT GCA AGC TAT ACC TAT ACA GTC TAT CGT GAC
 D M E V E D D S P A S Y T Y T V Y R D>

GCG ACG AAG ATC AAG GAA GGT CTG ACC GAA ACC ACC TAC CGC GAT GCA GGA ATG AGT
 G T K I K E G L T E T T Y R D A G M S>

5800

GCA CAA TCT CAT GAG TAT TGC GTA GAG GTT AAG TAC GCA GCC GGC GTC TCT CCG AAG
 A Q S H E Y C V E V K Y A A G V S P K>

GTT TGT GTG GAT TAT ATT CCT GAC GGA GTG GCA GAC GTC ACG GCT CAG AAG CCT TAC
 V C V D Y I P D G V A D V T A Q K P Y>

5900

ACG CTG ACA GTT GTT GGA AAG ACG ATC ACG GTA ACT TGC CAA GGC GAA GCT ATG ATC
 T L T V V G K T I T V T C Q G E A M I>

TAC GAC ATG AAC GGT CGT CGT CTG GCA GCC GGT CGC AAC ACA GTC GTT TAC ACG GCT
 Y D M N G R R L A A G R N T V V Y T A>

6000

CAG GGC GGC TAC TAT GCA GTC ATG GTT GTC GTT GAC GGC AAG TCT TAC GTC GAG AAA
 Q G G Y Y A V M V V V D G K S Y V E K>

6100

CTC GCT GTA AAG TAA TTCTGTC TTGGACTCGG AGACTTTGTG CAGACACTTT TAATATAGGT
 L A V K >

>Clal

CTGTAATTGT CTCAGAGTAT GAATCGATCG CCCGACCTCC TTTTAAGGAA GTCTGGCGA CTTCGTTTTT

6200

ATGCCTATTA TTCTAATATA CTCTGAAAC AATTTGTTCC AAAAAGTTGC ATGAAAAGAT TATCTTACTA

6300

TCTTTGCACT GCAAAAGGGG AGTTTCTAA GGTTTCCCC GGAGTAGTAC GGTAAATAACG GTGTGGTAGT

>Pvu2

TCAGCTGGTT AGAATACCTG CCTGTCACCC AGGGGGTCCG GGGTTCCAGT CCCGTCATA CGCGTAAATA

6400

GCTGAAAGAT AGGCTATAGG TCATCTGAAG CAATTTAGA AACGAATCCA AAAGCGTCTT AATTCCAACG

TABLE 2 (cont'd)

35

6500

AATTAAGGCG CTTTTCTTT GTCGCCACCC CACACGTCGG ATGAGGTTCG GAATAGGCGT ATATTCCGTA

6600

AATATGCCTC CGGTGGTTC C ATTGGTTA CAAAAAACAA AGGGGCTGAA AATTGTAACC ACAGACGACG

>NdeI

TAAAGACGAT GTTTAGACGA TTGACAAATT ACTCTGTTTC AAAATCATAT GTCGAACCTT GTAGCCGTAT

6700

GGTTACACTA ATTTGGAGC AAAATGAAGA GTCAATTTCG TTCAGTTTT TACTTGCAGCA GCAATTACAT

6800

CAACAAAGAA CGTAAAACTC CTGTCCTTAT TCGTATTTAT CTGAATAAGG AACGCCTGTC GTTGGGTTCG

ACAGGGCTGG CTGTTAATCC CATAACAATGG GATTCAGAAA AAGAGAAAGT CAAAGGACAT AGTGCAGAAG

6900

CACTTGAAGT CAATCGAAAG ATCGAAGAAA TCAGGGCTGA TATTCTGACC ATTTACAAAC GTTTGGAAGT

7000

AACAGTAGAT GATTGACGC CGGAGAGGAT CAAATCGGAA TACTGCGGAC AGACGGATAC ATTAAACAGT

ATAGTGGAAC TTTTCGATAA ACATAACGAG GATGTCCGGG CCCAGGTGGG AATCAATAAA ACGGCTGCCA

7100

CTTTACAAAA ATACGAAAC AGCAACGGC ATTTTACCCG ATTCTCAAA GCGAAGTACA ACAGAACGGA

7200

TCTCAAATTTC TCAGAGCTTA CCCCGTTGGT CATTCTAAC TTTGAGATAT ATCTGCTGAC TGTAGCCCAT

>HindI

TGTTGCCCGA ATACGGCAAC CAAAATCTTG AAGCTT

Cleavage of the precursor protein after the Arg residue at amino acid 227 removes the N-terminal precursor portion and after the Arg residue at amino acid 719, 1091 and 1429 releases a low molecular weight Arg-gingipain and three hemagglutinin components. The 44 kDa hemagglutin component has an amino acid sequence as given in SEQ ID NO:11 from 720-1091, with calculated molecular weight of 39.4 kDa, consistent with that estimated by gel electrophoresis. The 17 kDa hemagglutinin component has an amino acid sequence as given in SEQ ID NO:11 at amino acids 1092-1429, and a calculated molecular weight of 37.1 kDa. The 27 kDa hemagglutinin component has an amino acid sequence extending from amino acids 1430-1704 in SEQ ID NO:11, and a calculated molecular weight of 29.6 kDa.

TABLE 3
Alignment of Hemagglutinin Domain Sequences shown in Fig.2.

10	RGP ¹	amino acids 670-674 of SEQ ID NO:11	Ltatt
	HGP-44kDa ²	amino acids 865-913 of SEQ ID NO:11	dYTtTVYRDGKIKEGLTaaTRfeedGatgnHEYCVEVKYtAGVSPKvC
	HGP-17kDa	amino acids 1320-1368 of SEQ ID NO:11	dYTtTVYRDGKIKEGLTe1TRfeedGatgnHEYCVEVKYtAGVSPKec
15	HGP-27kDa	amino acids 1580-1626 of SEQ ID NO:11	SYTYTVYRDGKIKEGLTe1TRyrdagmsaqSHYCVEVKYaaAGVSPKvC

1 RGP means Arg-gingipain proteolytic component

2 HGP means Hemagglutinin protein component

Table 3 is the result of sequence comparison of the 44 kDa, 27 kDa and 17 kDa hemagglutinin domains of Arg-gingipain complexes, alignment of regions of amino acid identity, which without wishing to be bound by any particular theory, are postulated to be the domains responsible for hemagglutinin activity. Identical amino acids among all hemagglutinin domains are in capital letters, and amino acids which are not conserved are shown in lower case letters. In the case of the proteolytic component, only a limited region with significant match is shown.

10

A genomic DNA library was also prepared from virulent P. gingivalis W50. Two clones were identified as containing Arg-gingipain coding sequence. 0.5 and 3.5 kb BamHI fragments were sequenced; it exhibited 99% nucleotide sequence identity with about 3160 plus 557 bp of P. gingivalis H66 DNA containing Arg-gingipain coding sequence. A comparison of the deduced amino acid sequences of the encoded Arg-gingipain sequences revealed 99% identity.

20

Tables 1 and 2 both represent sequences from P. gingivalis. However, it is understood that there will be some variations in the amino acid sequences and encoding nucleic acid sequences for Arg-gingipain from different P. gingivalis strains. The ordinary skilled artisan can readily identify and isolate Arg-gingipain-encoding sequences from other strains where there is at least 70% homology to the specifically exemplified sequences herein using the sequences provided herein taken with what is well known to the art. Also within the scope of the present invention are Arg-gingipain where the protease or proteolytic component has at least about 85% amino acid sequence identity with an amino acid sequence exemplified herein.

30

It is also understood by the skilled artisan that there can be limited numbers of amino acid substitutions in a protein without significantly affecting function, and that nonexemplified gingipain-1 proteins can have some amino acid sequence diversion from the exemplified amino acid sequence. Such naturally

occurring variants can be identified, e.g., by hybridization to the exemplified (mature) Arg-gingipain-2 coding sequence (or a portion thereof capable of specific hybridization to Arg-gingipain sequences) under conditions appropriate to detect at least about 70% nucleotide sequence homology, preferably about 80%, more preferably about 90% and most preferably 95-100% sequence homology. Preferably the encoded Arg-gingipain protease or proteolytic component has at least about 85% amino acid sequence identity to an exemplified Arg-gingipain amino acid sequence.

It is well known in the biological arts that certain amino acid substitutions can be made in protein sequences without affecting the function of the protein. Generally, conservative amino acids are tolerated without affecting protein function. Similar amino acids can be those that are similar in size and/or charge properties, for example, aspartate and glutamate and isoleucine and valine are both pairs of similar amino acids. Similarity between amino acid pairs has been assessed in the art in a number of ways. For example, Dayhoff et al. (1978) in *Atlas of Protein Sequence and Structure*, Volume 5, Supplement 3, Chapter 22, pages 345-352, which is incorporated by reference herein, provides frequency tables for amino acid substitutions which can be employed as a measure of amino acid similarity. Dayhoff et al.'s frequency tables are based on comparisons of amino acid sequences for proteins having the same function from a variety of evolutionarily different sources.

The skilled artisan recognizes that other P. gingivalis strains can have coding sequences for a protein with the distinguishing characteristics of an Arg-gingipain; those coding sequences may be identical to or synonymous with the exemplified coding sequence, or there may be some variation(s) in the encoded amino acid sequence. An Arg-gingipain coding sequence from a P. gingivalis strain other than H66 can be identified by, e.g. hybridization to a polynucleotide or an oligonucleotide having the whole or a portion of the exemplified coding sequence for

mature gingipain, under stringency conditions appropriate to detect a sequence of at least 70% homology.

5 A polynucleotide or fragment thereof is "substantially homologous" (or "substantially similar") to another polynucleotide if, when optimally aligned (with appropriate nucleotide insertions or deletions) with another polynucleotide, there is nucleotide sequence identity for approximately 60% of the nucleotide bases, usually approximately 70%, more usually 10 about 80%, preferably about 90%, and more preferably about 95% to 100% of the nucleotide bases.

15 Alternatively, substantial homology (or similarity) exists when a polynucleotide or fragment thereof will hybridize to another under polynucleotide under selective hybridization conditions. Selectivity of hybridization exists under hybridization conditions which allow one to distinguish the target polynucleotide of interest from other polynucleotides. Typically, selective hybridization will occur when there is 20 approximately 55% similarity over a stretch of about 14 nucleotides, preferably approximately 65%, more preferably approximately 75%, and most preferably approximately 90%. See Kanehisa (1984) *Nuc. Acids Res.*, 12:203-213. The length of 25 homology comparison, as described, may be over longer stretches, and in certain embodiments will often be over a stretch of about 17 to 20 nucleotides, and preferably about 36 or more nucleotides.

30 The hybridization of polynucleotides is affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing polynucleotides, as will be readily appreciated by those skilled in the art. Stringent 35 temperature conditions will generally include temperatures in excess of 30°C, typically in excess of 37°C, and preferably in excess of 45°C. Stringent salt conditions will ordinarily be

less than 1 M, typically less than 500 mM, and preferably less than 200 mM. However, the combination of parameters is much more important than the measure of any single parameter (Wetmur and Davidson (1968) *J. Mol. Biol.* 31, 349-370).

5

An "isolated" or "substantially pure" polynucleotide is a polynucleotide which is substantially separated from other polynucleotide sequences which naturally accompany a native gingipain-1 sequence. The term embraces a polynucleotide sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates, chemically synthesized analogues and analogues biologically synthesized by heterologous systems.

15 A polynucleotide is said to "encode" a polypeptide if, in its native state or when manipulated by methods known to those skilled in the art, it can be transcribed and/or translated to produce the polypeptide of a fragment thereof. The anti-sense strand of such a polynucleotide is also said to encode the sequence.

20 A nucleotide sequence is operably linked when it is placed into a functional relationship with another nucleotide sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression. Generally, operably linked means that the sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. However, it is well known that certain genetic elements, such as enhancers, may be 25 operably linked even at a distance, i.e., even if not contiguous.

30 The term "recombinant" polynucleotide refers to a polynucleotide which is made by the combination of two otherwise separated segments of sequence accomplished by the artificial manipulation of isolated segments of polynucleotides by genetic engineering techniques or by chemical synthesis. In so doing one

may join together polynucleotide segments of desired functions to generate a desired combination of functions.

Polynucleotide probes include an isolated polynucleotide attached to a label or reporter molecule and may be used to identify and isolate other Arg-gingipain coding sequences. Probes comprising synthetic oligonucleotides or other polynucleotides may be derived from naturally occurring or recombinant single or double stranded nucleic acids or be chemically synthesized. Polynucleotide probes may be labelled by any of the methods known in the art, e.g., random hexamer labeling, nick translation, or the Klenow fill-in reaction.

Large amounts of the polynucleotides may be produced by replication in a suitable host cell. Natural or synthetic DNA fragments coding for a proteinase or a fragment thereof will be incorporated into recombinant polynucleotide constructs, typically DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the construct will be suitable for replication in a unicellular host, such as yeast or bacteria, but a multicellular eukaryotic host may also be appropriate, with or without integration within the genome of the host cells. Commonly used prokaryotic hosts include strains of Escherichia coli, although other prokaryotes, such as Bacillus subtilis or Pseudomonas may also be used. Mammalian or other eukaryotic host cells include yeast, filamentous fungi, plant, insect, amphibian and avian species. Such factors as ease of manipulation, ability to appropriately glycosylate expressed proteins, degree and control of protein expression, ease of purification of expressed proteins away from cellular contaminants or other factors may determine the choice of the host cell.

The polynucleotides may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Caruthers (1981) Tetra. Letts., 22: 1859-1862 or the triester method according to Matteuci et al. (1981) J. Am. Chem.

Soc., 103: 3185, and may be performed on commercial automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the 5 strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

DNA constructs prepared for introduction into a prokaryotic 10 or eukaryotic host will typically comprise a replication system (i.e. vector) recognized by the host, including the intended DNA fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide-encoding 15 segment. Expression systems (expression vectors) may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, 20 transcriptional terminator sequences, and mRNA stabilizing sequences. Signal peptides may also be included where appropriate from secreted polypeptides of the same or related species, which allow the protein to cross and/or lodge in cell membranes or be secreted from the cell.

25

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host. Examples of workable combinations of cell lines and expression vectors are described in Sambrook et al. (1989) vide infra; Ausubel et al. 30 (Eds.) (1987) Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York; and Metzger et al. (1988) Nature, 334: 31-36. Many useful vectors for expression in bacteria, yeast, mammalian, insect, plant or other cells are well known in the art and may be obtained such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. 35 In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made.

For appropriate enhancer and other expression control sequences, see also Enhancers and Eukaryotic Gene Expression, Cold Spring Harbor Press, N.Y. (1983). While such expression vectors may replicate autonomously, they may less preferably replicate by being inserted into the genome of the host cell.

Expression and cloning vectors will likely contain a selectable marker, that is, a gene encoding a protein necessary for the survival or growth of a host cell transformed with the vector. Although such a marker gene may be carried on another polynucleotide sequence co-introduced into the host cell, it is most often contained on the cloning vector. Only those host cells into which the marker gene has been introduced will survive and/or grow under selective conditions. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxic substances; e.g., ampicillin, neomycin, methotrexate, etc.; (b) complement auxotrophic deficiencies; or (c) supply critical nutrients not available from complex media. The choice of the proper selectable marker will depend on the host cell; appropriate markers for different hosts are known in the art.

The recombinant vectors containing the Arg-gingipain coding sequences of interest can be introduced (transformed, transfected) into the host cell by any of a number of appropriate means, including electroporation; transformation or transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and transfection or infection (where the vector is an infectious agent, such as a viral or retroviral genome). The choice of such means will often depend on the host cell. Large quantities of the polynucleotides and polypeptides of the present invention may be prepared by transforming suitable prokaryotic or eukaryotic host cells with gingipain-1-encoding polynucleotides of the present invention in compatible vectors or other expression vehicles and culturing such transformed host cells under conditions suitable to attain expression of the Arg-

gingipain-encoding gene. The Arg-gingipain may then be recovered from the host cell and purified.

The coding sequence for the "mature" form of Arg-gingipain-2
5 is expressed after PCR site-directed mutagenesis and cloning into an expression vector suitable for use in E. coli, for example. Exemplary expression vectors for E. coli and other host cells are given, for example in Sambrook et al. (1989), vide infra, and in Pouwels et al. (Eds.) (1986) Cloning Vectors, Elsevier Science
10 Publishers, Amsterdam, the Netherlands.

In order to eliminate leader sequences and precursor sequences at the 5' side of the coding sequence, a combination of restriction endonuclease cutting and site-directed mutagenesis
15 via PCR using an oligonucleotide containing a desired restriction site for cloning (one not present in coding sequence), a ribosome binding site, an translation initiation codon (ATG) and the codons for the first amino acids of the mature Arg-gingipain-2. The oligonucleotide for site-directed mutagenesis at the 3' end
20 of the coding sequence for mature gingipain-1 includes nucleotides encoding the carboxyterminal amino acids of mature gingipain-1, a translation termination codon (TAA, TGA or TAG), and a second suitable restriction endonuclease recognition site not present in the remainder of the DNA sequence to be inserted
25 into the expression vector. The site-directed mutagenesis strategy is similar to that of Boone et al. (1990) Proc. Natl. Acad. Sci. USA 87: 2800-2804, as modified for use with PCR.

In another embodiment, polyclonal and/or monoclonal antibodies capable of specifically binding to a proteinase or fragments thereof are provided. The term antibody is used to refer both to a homogenous molecular entity, or a mixture such as a serum product made up of a plurality of different molecular entities. Monoclonal or polyclonal antibodies specifically reacting with the Arg-gingipains may be made by methods known in the art. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratories; Goding (1986)

5 Monoclonal Antibodies: Principles and Practice, 2d ed., Academic Press, New York; and Ausubel et al. (1987) supra. Also, recombinant immunoglobulins may be produced by methods known in the art, including but not limited to the methods described in U.S. Patent No. 4,816,567. Monoclonal antibodies with affinities of 10^8 M $^{-1}$, preferably 10^9 to 10^{10} or more are preferred.

10 Antibodies specific for Arg-gingipains may be useful, for example, as probes for screening DNA expression libraries or for detecting the presence of Arg-gingipains in a test sample. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or noncovalently, a substance which provides a detectable signal. Suitable labels include but are not limited to radionuclides, enzymes, substrates, cofactors, 15 inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. United States Patents describing the use of such labels include but are not limited to Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

20

Antibodies specific for Arg-gingipain(s) and capable of inhibiting its proteinase activity may be useful in treating animals, including man, suffering from periodontal disease. Such antibodies can be obtained by the methods described above and 25 subsequently screening the Arg-gingipain-specific antibodies for their ability to inhibit proteinase activity.

30 Compositions and immunogenic preparations including vaccine compositions comprising substantially purified recombinant Arg-gingipain(s) and a suitable carrier therefor are provided. Alternatively, hydrophilic regions of the proteolytic component or hemagglutinin component(s) of Arg-gingipain can be identified by the skilled artisan, and peptide antigens can be synthesized and conjugated to a suitable carrier protein (e.g., bovine serum albumin or keyhole limpet hemocyanin) for use in vaccines or in 35 raising antibody specific for Arg-gingipains. Immunogenic compositions are those which result in specific antibody

production when injected into a human or an animal. Such vaccines are useful, for example, in immunizing an animal, including humans, against inflammatory response and tissue damage caused by *P. gingivalis* in periodontal disease. The vaccine 5 preparations comprise an immunogenic amount of one or more Arg-gingipains or an immunogenic fragment(s) or subunit(s) thereof. Such vaccines may comprise one or more Arg-gingipain proteinases, or in combination with another protein or other immunogen. By "immunogenic amount" is meant an amount capable of eliciting the 10 production of antibodies directed against Arg-gingipain(s) in an individual to which the vaccine has been administered.

Immunogenic carriers may be used to enhance the 15 immunogenicity of the proteinases. Such carriers include but are not limited to proteins and polysaccharides, liposomes, and bacterial cells and membranes. Protein carriers may be joined to the proteinases to form fusion proteins by recombinant or synthetic means or by chemical coupling. Useful carriers and 20 means of coupling such carriers to polypeptide antigens are known in the art.

The vaccines may be formulated by any of the means known in 25 the art. Such vaccines are typically prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also, for example, be emulsified, or the protein encapsulated in liposomes.

The active immunogenic ingredients are often mixed with 30 excipients or carriers which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients include but are not limited to water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. The concentration of the immunogenic polypeptide in injectable formulations is 35 usually in the range of 0.2 to 5 mg/ml.

In addition, if desired, the vaccines may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may 5 be effective include but are not limited to: aluminum hydroxide; N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP); N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP); N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3hydroxyphosphoryloxy)-ethylamine 10 (CGP 19835A, referred to as MTP-PE); and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies 15 directed against the immunogen resulting from administration of the immunogen in vaccines which are also comprised of the various adjuvants. Such additional formulations and modes of administration as are known in the art may also be used.

20 50 kDa Arg-gingipain or high molecular weight Arg-gingipain and fragments thereof may be formulated into vaccines as neutral or salt forms. Pharmaceutically acceptable salts include but are not limited to the acid addition salts (formed with free amino groups of the peptide) which are formed with inorganic acids, 25 e.g., hydrochloric acid or phosphoric acids; and organic acids, e.g., acetic, oxalic, tartaric, or maleic acid. Salts formed with the free carboxyl groups may also be derived from inorganic bases, e.g., sodium, potassium, ammonium, calcium, or ferric hydroxides, and organic bases, e.g., isopropylamine, trimethylamine, 2-ethylamino-ethanol, histidine, and procaine.

30 The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of about 35 100 to 1,000 µg of protein per dose, more generally in the range of about 5 to 500 µg of protein per dose, depends on the subject to

be treated, the capacity of the individual's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of the active ingredient required to be administered may depend on the judgment of the physician or doctor of dental medicine and may be peculiar to each individual, but such a determination is within the skill of such a practitioner.

The vaccine or other immunogenic composition may be given in a single dose or multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may include 1 to 10 or more separate doses, followed by other doses administered at subsequent time intervals as required to maintain and/or reinforce the immune response, e.g., at 1 to 4 months for a second dose, and if needed, a subsequent dose(s) after several months.

Recombinant Arg-gingipains are useful in methods of identifying agents that modulate proteinase activity, e.g., by acting on the proteinase itself. One such method comprises the steps of incubating Arg-gingipain-1 (or high molecular weight Arg-proteinase) with a putative therapeutic agent; determining the activity of the proteinase incubated with the agent; and comparing the activity obtained in step with the activity of a control sample of proteinase that has not been incubated with the agent.

All references cited herein are hereby incorporated by reference in their entirety.

Except as noted hereafter, standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) Molecular Cloning, Second Edition, Cold Spring Harbor

Laboratory, Plainview, New York; Maniatis et al. (1982) Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) Meth. Enzymol. 218, Part I; Wu (ed.) (1979) Meth Enzymol. 68; Wu et al. (eds.) (1983) Meth. Enzymol. 100 and 101; 5 Grossman and Moldave (eds.) Meth. Enzymol. 65; Miller (ed.) (1972) Experiments in Molecular Genetics, Cold spring Harbor Laboratory, Cold Spring Harbor, New York, Old Primrose (1981) Principles of Gene Manipulation, University of California Press, Berkeley; Schleif and Wensink (1982) Practical Methods in 10 Molecular Biology; Glover (ed.) (1985) DNA Cloning Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) Nucleic Acid Hybridization, IRL Press, Oxford, UK; Setlow and Hollaender (1979) Genetic Engineering: Principles and Methods, Vols. 1-4, 15 Plenum Press, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

The foregoing discussion and the following examples illustrate but are not intended to limit the invention. The skilled artisan will understand that alternative methods may be used to implement the invention.

EXAMPLES

25 Example 1 Purification of Gingipain Enzymes

Example 1.1 Bacterial Cultivation

30 P. gingivalis strains H66 (ATCC 33277) and W50 (ATCC 53978) (virulent) were used in these studies. Cells were grown in 500 ml of broth containing 15.0 g Trypticase Soy Broth (Difco, Detroit, Michigan), 2.5 g yeast extract, 2.5 mg hemin, 0.25 g cysteine, 0.05 g dithiothreitol, 0.5 mg menadione (all from Sigma Chemical Company, St. Louis, MO) anaerobically at 37°C for 48 hr in an atmosphere of 85% N₂, 10% CO₂, 5% H₂. The entire 500 ml culture was used to inoculate 20 liters of the same medium, and the latter was incubated in a fermentation tank at 37°C for 48 hr (to a final optical density of 1.8 at 650 nm).

Example 1.2 Purification of Low Molecular Weight Arg-gingipain

1200 ml cell-free supernatant was obtained from the 48 hr culture by centrifugation at 18,000 x g for 30 min. at 4°C. Proteins in the supernatant were precipitated out by 90% saturation with ammonium sulfate. After 2 hr at 4°C, the suspension was centrifuged at 18,000 x g for 30 min. The resulting pellet was dissolved in 0.05 M sodium acetate buffer, pH 4.5, 0.15 NaCl, 5 mM CaCl₂; the solution was dialyzed against the same buffer overnight at 4°C, with three changes with a buffer:protein solution larger than 150:1. The dialysate was then centrifuged at 25,000 x g for 30 min., and the dark brown supernatant (26 ml) was then chromatographed over an agarose gel filtration column (5.0 x 150 cm; Sephadex G-150, Pharmacia, Piscataway, NJ) which had been pre-equilibrated with the same buffer. The column was developed with said buffer at a flow rate of 36 ml/hr. 6 ml fractions were collected and assayed for both amidolytic and proteolytic activities, using Bz-L-Arg-pNA and azocasein as substrates. Four peaks containing amidolytic activity were identified (Fig. 1). The fractions corresponding to peak 4 were combined, concentrated by ultrafiltration (Amicon PM-10 membrane; Amicon, Beverly, MA) and then dialyzed overnight against 0.05 Bis-Tris, 5 mM CaCl₂, pH 6.0. The volume of the dialysate was 14 ml.

The 14 ml dialysate from the previous step was then applied to a DEAE-cellulose (Whatman, Maidstone, England) column (1 x 10 cm) equilibrated with 0.05 mM Bis-Tris, 5 mM CaCl₂, pH 6.0. The column was then washed with an additional 100 ml of the same buffer. About 75% of the amidolytic activity, but only about 50% of the protein, passed through the column. The column wash fluid was dialyzed against 0.05 M sodium acetate buffer containing 5 mM CaCl₂ (pH 4.5). This 19 ml dialysate was applied to a Mono S FPLC column (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) equilibrated with the same buffer. The column was washed with the starting buffer at a flow rate of 1.0 ml/min for 20 min. Bound proteins were eluted first with a linear NaCl gradient (0 to 0.1 M) followed by a second linear NaCl gradient (0.1 to 0.25 M).

M), each gradient applied over a 25 min time period. Fractions were assayed for amidolytic activity using Bz-L-Arg-pNA. Fractions with activity were pooled and re-chromatographed using the same conditions. Although not detectable by gel electrophoresis, trace contamination by a proteinase capable of cleaving after lysyl residues was sometimes observed. This contaminating activity was readily removed by applying the sample to an arginyl-agarose column (L-Arginyl-SEPHAROSE 4B) equilibrated with 0.025 M Tris-HCl, 5 mM CaCl₂, 0.15 M NaCl, pH 7.5. After washing with the same buffer, purified enzyme was eluted with 0.05 M sodium acetate buffer, 5 mM CaCl₂, pH 4.5. Yields of gingipain-1 were markedly reduced by this step (about 60%).

15 Example 1.3 High Molecular Weight Arg-gingipain Purification

The culture supernatant (2,900 ml) was obtained by centrifugation of the whole culture (6,000 x g, 30 min, 4°C). Chilled acetone (4,350 ml) was added to this fraction over a period of 15 min, with the temperature of the solution maintained below 0°C at all times, using an ice/salt bath and this mixture was centrifuged (6,000 x g, 30 min, -15°C). The precipitate was dissolved in 290 ml of 20 mM Bis-Tris-HCl, 150 mM NaCl, 5 mM CaCl₂, 0.02% (w/v) NaN₃, pH 6.8 (Buffer A), and dialyzed against Buffer A containing 1.5 mM 4,4'-Dithiodipyridine disulfide for 4h, followed by 2 changes of buffer A overnight. The dialyzed fraction was centrifuged (27,000 x g, 30 min, 4°C), following which it was concentrated to 40 ml by ultrafiltration using an Amicon PM-10 membrane. This concentrated fraction was applied to a Sephadex G-150 column (5 x 115 cm = 2260 ml; Pharmacia, Piscataway, NJ) which had previously been equilibrated with Buffer A, and the fractionation was carried out at 30 ml/h (1.5 cm/h). Fractions (9 ml) were assayed for activity against Bz-L-Arg-pNa and Z-L-Lys-pNa (Novabiochem; 0.5 mM). Amidolytic activities for Bz-L-Arg-pNa (0.5 mM) or Z-L-Lys-pNa were measured in 0.2 M Tris-HCl, 1 mM CaCl₂, 0.02% (w/v) NaN₃, 10 mM L-cysteine, pH 7.6. General proteolytic activity was measured with azocasein (2% w/v) as described by Barrett and Kirschke (1981) Meth.

Enzymol. 80, 535-561 for cathepsin L. Three peaks with activity against the two substrates were found. The first (highest molecular weight) peak of activity was pooled, concentrated to 60 ml using ultrafiltration and dialyzed overnight against two changes of 50 mM Tris-HCl, 1 mM CaCl₂, 0.02% NaN₃, pH 7.4 (Buffer B).

This high MW fraction was applied to an L-Arginine-Sepharose column (1.5 x 30 cm = 50 ml), which had previously been equilibrated with Buffer B at a flow rate of 20 ml/hr (11.3 cm/h), following which the column was washed with two column volumes of Buffer B. Following this, a step gradient of 500 mM NaCl was applied in Buffer B and the column was washed with this concentration of NaCl until the A₂₈₀ baseline fell to zero. After re-equilibration of the column in Buffer B, a gradient from 0-750 mM L-Lysine was applied in a total volume of 300 ml, followed by 100 ml of 750 mM L-Lysine. The column was once again re-equilibrated with Buffer B and a further gradient to 100 mM L-arginine in 300 ml was applied in the same way. Fractions (6 ml) from the Arg wash were assayed for activity against the two substrates as described previously. The arginine gradient eluted a major peak for an enzyme degrading Bz-L-Arg-pNa. The active fractions were pooled and dialyzed against two changes of 20 mM Bis-Tris-HCl, 1 mM CaCl₂, 0.02% (v/w) NaN₃, pH 6.4 (Buffer C) and concentrated down to 10 ml using an Amicon PM-10 membrane.

The concentrate with activity for cleaving Bz-L-Arg-pNa was applied to a Mono Q FPLC column (Pharmacia LKB Biotechnology Inc, Piscataway, NJ) equilibrated in Buffer C, the column was washed with 5 column volumes of Buffer C at 1.0 ml/min, following which bound protein was eluted with a 3 step gradient [0-200 mM NaCl (10 min), followed by 200-250 mM NaCl (15 min) and 250-500 mM NaCl (5 min)]. The active fractions from Mono Q were pooled and used for further analyses.

Example 2 Molecular Weight Determination

The molecular weight of the purified Arg-gingipain-1 was estimated by gel filtration on a Superose 12 column (Pharmacia, Piscataway, NJ) and by Tricine-SDS polyacrylamide gel electrophoresis. In the latter case, 1 mM TLCK was used to inactivate the protease prior to boiling, thus preventing autoproteolytic digestion.

Example 3 Enzyme Assays

Amidolytic activities of *P. gingivalis* proteinases were measured with the substrates MeO-Suc-Ala-Ala-Pro-Val-pNA at a concentration of 0.5 mM, Suc-Ala-Ala-Ala-pNA (0.5 mM), Suc-Ala-Ala-Pro-Phe-pNA (0.5 mM), Bz-Arg-pNA (1.0 mM), Cbz-Phe-Leu-Glu-pNA (0.2 mM); S-2238, S-2222, S-2288 and S-2251 each at a concentration of 0.05 mM; in 1.0 ml of 0.2 M Tris-HCl, 5 mM CaCl₂, pH 7.5. In some cases either 5 mM cysteine and/or 50 mM glycyl-glycine (Gly-Gly) was also added to the reaction mixture.

For routine assays, pH optimum determination and measurement of the effect of stimulating agents and inhibitors on trypsin-like enzymes, only Bz-L-Arg-pNA was used as substrate. Potential inhibitory or stimulatory compounds were preincubated with enzyme for up to 20 min at room temperature at pH 7.5, in the presence of 5 mM CaCl₂ (except when testing the effects of chelating agents) prior to the assay for enzyme activity.

General proteolytic activity was assayed using the same buffer system as described for detecting amidolytic activity, but using azocoll or azocasein (1% w/v) as substrate.

A unit of Arg-gingipain-1 enzymatic activity is based on the spectroscopic assay using benzoyl-Arg-p-nitroanilide as substrate and recording Δ absorbance units at 405 nm/min/absorbance unit at 280 nm according to the method of Chen et al. (1992) supra.

Example 4 Enzyme Specificity

Purified Arg-gingipain-1 (0.8 µg) in 50 mM ammonium bicarbonate buffer, pH 7.7, 5 mM CaCl₂, was preincubated with 2 mM cysteine for 10 min, followed by the addition of either 5 oxidized insulin B chain (225 µg) or melittin (225 µg) at 25°C. Samples were removed after various time intervals, and the reaction mixtures were subjected to HPLC (reverse phase column, MicroPak SP C-18 column) using linear gradients (0.08% 10 trifluoroacetic acid to 0.08% trifluoroacetic acid plus 80% acetonitrile, over a 45 min period (flow rate 1.0 ml/min). Peptides were detected by monitoring A₂₂₀. Product peaks were collected and subjected to amino acid analysis and/or amino-terminal sequence analysis.

Example 5 Amino Acid Sequence Analysis

Amino-terminal amino acid sequence analysis of either Arg-gingipain-1 or degradation products from proteolytic reactions was carried out using an Applied Biosystems 4760A gas-phase sequenator, using the program designed by the manufacturer.

The amino acid sequence of the COOH terminus of SDS-denatured Arg-gingipain-1 and of Arg-gingipain-2 was determined. 20 10 nmol aliquots of gingipain-1 were digested in 0.2 M N-ethylmorpholine acetate buffer, pH 8.0, with carboxypeptidase A and B at room temperature, using 1:100 and 1:50 molar ratios, respectively. Samples were removed at intervals spanning 0 to 12 hours, boiled to inactivate the carboxypeptidase, and protein 25 was precipitated with 20% trichloroacetic acid. Amino acid analysis was performed on the supernatants.

Example 6 Materials

MeO-Suc-Ala-Ala-Pro-Val-pNA, Suc-Ala-Ala-Pro-Phe-pNA, Gly-Pro-pNA, Suc-Ala-Ala-Ala-pNA, Bz-Arg-pNA, diisopropylfluorophosphate, phenylmethylsulfonyl fluoride, tosyl-L-lysine chloromethyl ketone (TLCK), tosyl-L-phenylalanine chloromethyl ketone (TPCK), *trans*-epoxysuccinyl-L-leucylamide-(4-guanidino)butane), an inhibitor of cysteine proteinases,

leupeptin, antipain and azocasein were obtained from Sigma Chemical Co., St. Louis, MO. 3,4-Dichloroisocoumarin was obtained from Boehringer, Indianapolis, IN and CBz-Phe-Leu-Glu-pNA and azocoll were obtained from Calbiochem, La Jolla, CA. S-
5 2238 (D-Phe-Pip-Arg-pNA), S-2222 (Bz-Ile-Glu-(γ -OR)-Gly-Arg-pNA), S-2288 (D-Ile-Pro-Arg-pNA), and S-2251 (D-Val-Leu-Lys-pNA) were from Kabi-Vitrum, (Beaumont, Texas).

Example 7 Electrophoresis

10 SDS-PAGE of Arg-gingipain-1 was performed as in Laemmli (1970) *Nature* 227: 680-685. Prior to electrophoresis the samples were boiled in a buffer containing 20% glycerol, 4% SDS, and 0.1% bromphenol blue. The samples were run under reducing conditions by adding 2% β -mercaptoethanol unless otherwise noted. Samples
15 were heated for 5 min at 100°C prior to loading onto gels. A 5-15% gradient gel was used for the initial digests of C3 and C5, and the gels were subsequently stained with Coomassie Brilliant Blue R. The C5 digest used to visualize breakdown products before and after reduction of the disulfide bonds were
20 electrophoresed in a 8% gel. Attempts to visualize C5a in the C5 digest were carried out using 13% gels that were developed with silver stain according to the method of Merril et al. (1979) *Proc. Natl. Acad. Sci. USA* 76, 4335-4340.

25 In some experiments (high molecular weight forms) SDS-PAGE using Tris-HCl/Tricine buffer was carried out per Shagger and Van Jagow (1987) *Analyt. Biochem.* 166, 368-379.

30 Electrophoresis on cellulose acetate strips were performed in 0.075 barbital buffer at pH 8.5 and 4°C for 30 min. at 200 V. The Beckman Microzone apparatus (model R101) used for the electrophoresis of the protein, and the strips were stained using Amido Black.

35 Example 8 Oligonucleotide Synthesis

Oligonucleotide primers for PCR probes and sequencing were synthesized by the phosphoramidite method with an Applied

Biosystems model 394 automated DNA synthesizer (Applied Biosystems, Foster City, CA) and purified by PAGE and desalted on Sep-Pak (Millipore Corp., Beverly, MA) using standard protocols. Primer GIN-1-32 was designed to bind to the noncoding strand of Arg-gingipain DNA corresponding to the NH₂-terminal portion of the mature protein, i.e., to the sequence encoding amino acids 2-8 within SEQ ID NO:1. The sequence of the 32-base primer consists of 20 bases specific for Arg-gingipain and six additional bases at the 5' end (underlined), as follows: 5'-GGCTTTACNCCNGTNGARGARYTNGA-3' (SEQ ID NO:6), where N is A or G or C or T. Primer GIN-2-30 was designed to bind to the coding strand of Arg-gingipain DNA corresponding to the amino acids 25-32 of the mature protein, i.e., residues 25-32 of SEQ ID NO:1. The sequence of the 30-base primer consists of 24 bases specific for gingipain-1 (and gingipain-2) DNA and six additional bases at the 5' end (underlined), as follows: 5'-GGCTTRTTYTTCCARTC NACRAARTCYTT-3', where R is A or G, Y is C or T and N is A or G or C or T (SEQ ID NO:7). Primer GIN-8S-48: 5'-CCTGGAGAATTCTCG TATGATCGTCATCGTAGCCAAAAAGTATGAGGG-3' (SEQ ID NO:8) was designed to bind to the noncoding strand of Arg-gingipain DNA corresponding to the amino acids 11-22 of the mature protein, i.e., amino acids 11-22 of SEQ ID NO:1, and was designed on the basis of partial DNA sequence information for the Arg-gingipain coding sequence (nucleotides 1659-1694 of SEQ ID NO:3) and included a 6-base ECORI restriction site plus six additional bases at the 5' end (underlined). This primer was used as a probe to screen a λDASH P. gingivalis genomic DNA library (see below). One additional oligonucleotide GIN-14-20 (20-mers), initially designed to sequence Arg-gingipain DNA, was used as a probe to identify and then clone the 3' end of the gingipain-1 coding sequence, as a PstI-HindIII sequence. Primer GIN-14-20 was designed to bind to the noncoding strand of gingipain-1 DNA corresponding to 20 bases specific for 3' end of Arg-gingipain (nucleotides 2911-2930 within SEQ ID NO:3): 5'-ATCAACACTAATGGTGAGCC-3' (SEQ ID NO:9). A total of 71 20-mers internal primers were designed using empirically determined sequence to sequence the Arg-gingipain locus.

Example 9 Polymerase Chain Reaction

The DNA templates used in PCR was P. gingivalis strain H66 total cellular DNA. The PCR was run using primer GIN-1-32 (SEQ ID NO:6) along with primer GIN-2-30 (SEQ ID NO:7); PCR 5 consistently yielded a single 105-base pair product (P105) detected on a 7% acrylamide gel representing a partial gingipain DNA. After treatment with the Klenow enzyme, P105 was cloned in pCR-ScriptTMSK(+) (Stratagene La Jolla, CA). After sequence analysis of P105, specific primer GIN-8S-48 (SEQ ID NO:8) was 10 designed to use as a probe. The ³²P-labeled GIN-8S-48 probe, was generated by kinase reaction for use in subsequent hybridization screening of the λDASH library. Incorporated nucleotides were separated from unincorporated nucleotides on a Sephadex G-25 column (Boehringer Mannheim Corporation, Indianapolis, IN).

15

Example 10 Construction and Screening of the genomic DNA library

λDASH and λZAP DNA libraries were constructed according to the protocols of Stratagene, using the lambda DASHTM II/BamHI cloning kit and DNA preparations from P. gingivalis strains H66 20 and W50. Libraries of 3×10^5 independent recombinant clones was obtained using P. gingivalis H66 DNA, and 1.5×10^5 independent recombinant clones were obtained from P. gingivalis W50 DNA.

Approximately 3×10^5 phages were grown on 5x150 mm agar 25 plates, lifted in duplicate onto supported nitrocellulose transfer membrane (BAS-NC, Schleicher & Schuell, Keene, NH), hybridized to the ³²P-labeled GIN-8S-48 probe described above. Hybridizations were performed overnight at 42°C in 2X Denhardt's solution (Denhardt, D.T. (1966), *Biochem. Biophys. Res. Comm.* 23, 30 641-646), 6X SSC (SSC is 15 mM sodium citrate, 150 mM NaCl), 0.4% SDS (w/v), 500 µg/ml fish sperm DNA. The filters were washed in 2X SSC containing 0.05% SDS (w/v) at 48°C. Seven positively hybridizing plaques were purified. After extraction and purification, the DNA was analyzed by restriction enzyme 35 digestion and agarose gel electrophoresis. The 3 kb-PstI fragment from clone A1 (P. gingivalis H66) was subsequently cloned into pBluescript SK(-) (Stratagene, La Jolla, CA) and

M13mp18 and 19 and sequenced. After restriction analysis of the A1 clone, a SmaI/BamHI fragment was then cloned into pBluescript SK(-). A PstI/BamHI smaller fragment was subcloned into M13mp18 and 19 for sequencing purposes. 3.5 and 0.5 kb-BamHI fragments from the λZAP P. gingivalis W50 DNA library were cloned into pBluescript SK(-) and M13mp18 and 19 and sequenced. Standard protocols for cDNA library screening, lambda phage purification, agarose gel electrophoresis and plasmid cloning were employed (Maniatis et al. (1982), supra). Standard protocols for cDNA library screening, lambda phage purification, agarose gel electrophoresis and plasmid cloning were employed (Maniatis et al., 1982 supra).

Example 11 Southern Blot Analysis

The membranes were washed as described above. BamHI, HindIII- or PstI-digested P. gingivalis H66 DNA samples were hybridized with ³²P-labeled GIN-8S-48. Two BamHI fragments of approximately 9.4 and 3.5 kb, and two PstI fragments of approximately 9.4 and 3 kb were found. No HindIII fragment was seen. BamHI- and PstI-digested λDASH DNA after screening and purification of positive recombinant clones from the library revealed one clone (A1) with a 3.5 kb BamHI fragment and a 3 kb PstI fragment; one clone (B1) with a 9.4 kb BamHI fragment and a 9.4 kb PstI fragment; and 5 clones with a 9.4 kb BamHI fragment and a 10 kb PstI fragment. The A1 clone was sequenced because the DNA predicted to encode a 50-kDa protein is approximately 1.35 kb. In order to clone the stop codon of Arg-gingipain-2, double PstI/HindIII-digested P. gingivalis DNA were hybridized with ³²P-labeled GIN-14-20. One PstI/HindIII fragment of approximately 4.3 kb was found. This fragment was gel purified and cloned into pBluescript SK(-) for sequencing. Smaller fragments (PstI/SmaI and BamHI/HindIII) were also subcloned into M13mp18 and 19 and sequenced, and was found to include the stop codon. Table 2 hereinabove (see also SEQ ID NO:10) which presents about 7 kb of sequence extending from a PstI site upstream of the start codon through a HindIII site downstream of the end of the prepolypeptide's stop codon.

Example 12 DNA Sequencing

Double-stranded DNA cloned into pBluescript SK(-) and single-stranded DNA cloned into M13mp18 and 19 were sequenced by the dideoxy terminator method [Sanger et al. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463-5467] using sequencing kits purchased from United States Biochemicals (Cleveland, OH; Sequenase version 2.0). The DNA was sequenced using M13 universal primer, reverse sequencing primer and internal primers as well understood in the art.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: UNIVERSITY OF GEORGIA, RESEARCH FOUNDATION INC.

(ii) TITLE OF INVENTION: Porphyromonas Gingivalis
Arginine-Specific Proteinase Coding Sequences

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Greenlee and Winner, P.C.
(B) STREET: 5370 Manhattan Circle, Suite 201
(C) CITY: Boulder
(D) STATE: CO
(E) COUNTRY: USA
(F) ZIP: 80303

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: Unassigned
(B) FILING DATE: 09-SEP-1994
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/119,361
(B) FILING DATE: 10-SEP-1993

(viii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/265,441
(B) FILING DATE: 24-JUN-1994

(ix) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/141,324
(B) FILING DATE: 21-OCT-1993

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(C) REFERENCE/DOCKET NUMBER: 21-93B PCT

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Porphyromonas gingivalis
- (B) STRAIN: H66

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Tyr Thr Pro Val Glu Glu Lys Gln Asn Gly Arg Met Ile Val Ile Val
1 5 10 15

Ala Lys Lys Tyr Glu Gly Asp Ile Lys Asp Phe Val Asp Trp Lys Asn
20 25 30

Gln Arg Gly Leu Thr Lys Xaa Val Lys Xaa Ala
35 40

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gly Tyr Gly Asp Ser Asn Tyr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3159 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 949..3159

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 1630..3105

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTGCAGAGGG CTGGTAAAGA CCGCCTCGGG ATCGAGGCCT TTGAGACGGG CACAAGCCGC	60
CGCAGCCTCC TCTTCGAAGG TGTCTCGAAC GTCCACATCG GTGAATCCGT AGCAGTGCTC	120
ATTGCCATTG AGCAGCACCG AGGTGTGGCG CATCAGATAT ATTTTCATCA GTGGATTATT	180
AGGGTATCGG TCAGAAAAAG CCTTCGAAT CCGACAAAGA TAGTAGAAAG AGAGTGCATC	240
TGAAAACAGA TCATTCGAGG ATTATCGATC AACTGAAAAG GCAGGGAGTTG TTTTGCCTT	300
TGGTTCCGAA AATTACCTGA TCAGCATTG TAAAAACGTG GCGCGAGAAT TTTTCGTTT	360
TGGCGCGAGA ATTAAAAATT TTTGGAACCA CAGCGAAAAA AATCTCGCGC CGTTTTCTCA	420
GGATTTACAG ACCACAATCC GAGCATTTC GGTTCGTAAT TCATCGAAGA GACAGGTTT	480
ACCGCATTGA AATCAGAGAG AGAATATCCG TAGTCCAACG GTTCATCCTT ATATCAGAGG	540
TTAAAAGATA TGGTACGCTC ATCGAGGAGC TGATTGGCTT AGTAGGTGAG ACTTTCTAA	600
GAGACTATCG GCACCTACAG GAAGTTCATG GCACACAAAGG CAARGGAGGC AATCTTCGCA	660
GACCGGACTC ATATCAAAGA GATGAAACGA CTTTCCATA CGACAACCAA ATAGCCGTCT	720
ACGGTAGACG AATGCAAACC CAATATGAGG CCATCAATCA ATCCGAATGA CAGCTTTGG	780
GCAATATATT ATGCATATT TGATTCGCGT TTAAAGGAAA AGTCATATA TTTGCGATTG	840
TGGTATTCTTCT TTCGGTTCT ATGTGAATT TGTCTCCCAA GAAGACTTTA TAATGCATAA	900
ATACAGAAGG GGTACTACAC AGTAAAATCA TATTCTAATT TCATCAAA ATG AAA AAC Met Lys Asn -227 -225	957
 TTG AAC AAG TTT GTT TCG ATT GCT CTT TGC TCT TCC TTA TTA GGA GGA Leu Asn Lys Phe Val Ser Ile Ala Leu Cys Ser Ser Leu Leu Gly Gly -220 -215 -210	1005
 ATG GCA TTT GCG CAG CAG ACA GAG TTG GGA CGC AAT CCG AAT GTC AGA Met Ala Phe Ala Gln Gln Thr Glu Leu Gly Arg Asn Pro Asn Val Arg -205 -200 -195	1053
 TTG CTC GAA TCC ACT CAG CAA TCG GTG ACA AAG GTT CAG TTC CGT ATG Leu Leu Glu Ser Thr Gln Gln Ser Val Thr Lys Val Gln Phe Arg Met -190 -185 -180	1101
 GAC AAC CTC AAG TTC ACC GAA GTT CAA ACC CCT AAG GGA ATC GGA CAA Asp Asn Leu Lys Phe Thr Glu Val Gln Thr Pro Lys Gly Ile Gly Gln -175 -170 -165	1149
 GTG CCG ACC TAT ACA GAA GGG GTT AAT CTT TCC GAA AAA GGG ATG CCT Val Pro Thr Tyr Thr Glu Gly Val Asn Leu Ser Glu Lys Gly Met Pro -160 -155 -150 -145	1197
 ACG CTT CCC ATT CTA TCA CGC TCT TTG GCG GTT TCA GAC ACT CGT GAG Thr Leu Pro Ile Leu Ser Arg Ser Leu Ala Val Ser Asp Thr Arg Glu -140 -135 -130	1245
 ATG AAG GTA GAG GTT GTT TCC TCA AAG TTC ATC GAA AAG AAA AAT GTC Met Lys Val Glu Val Val Ser Ser Lys Phe Ile Glu Lys Lys Asn Val -125 -120 -115	1293
 CTG ATT GCA CCC TCC AAG GCC ATG ATT ATG CGT AAC GAA GAT CCG AAA Leu Ile Ala Pro Ser Lys Gly Met Ile Met Arg Asn Glu Asp Pro Lys -110 -105 -100	1341

AAG ATC CCT TAC GTT TAT GGA AAG AGC TAC TCG CAA AAC AAA TTC TTC Lys Ile Pro Tyr Val Tyr Gly Lys Ser Tyr Ser Gln Asn Lys Phe Phe -95 -90 -85	1389
CCG GGA GAG ATC GCC ACG CTT GAT GAT CCT TTT ATC CTT CGT GAT GTG Pro Gly Glu Ile Ala Thr Leu Asp Asp Pro Phe Ile Leu Arg Asp Val -80 -75 -70 -65	1437
CGT GGA CAG GTT GTA AAC TTT GCG CCT TTG CAG TAT AAC CCT GTG ACA Arg Gly Gln Val Val Asn Phe Ala Pro Leu Gln Tyr Asn Pro Val Thr -60 -55 -50	1485
AAG ACG TTG CGC ATC TAT ACG GAA ATC ACT GTG GCA GTG AGC GAA ACT Lys Thr Leu Arg Ile Tyr Thr Glu Ile Thr Val Ala Val Ser Glu Thr -45 -40 -35	1533
TCG GAA CAA GGC AAA AAT ATT CTG AAC AAG AAA GGT ACA TTT GCC GGC Ser Glu Gln Gly Lys Asn Ile Leu Asn Lys Lys Gly Thr Phe Ala Gly -30 -25 -20	1581
TTT GAA GAC ACA TAC AAG CGC ATG TTC ATG AAC TAC GAG CCG GGG CGT Phe Glu Asp Thr Tyr Lys Arg Met Phe Met Asn Tyr Glu Pro Gly Arg -15 -10 -5	1629
TAC ACA CCG GTA GAG GAA AAA CAA AAT GGT CGT ATG ATC GTC ATC GTA Tyr Thr Pro Val Glu Lys Gln Asn Gly Arg Met Ile Val Ile Val 1 5 10 15	1677
GCC AAA AAG TAT GAG GGA GAT ATT AAA GAT TTC GTT GAT TGG AAA AAC Ala Lys Tyr Glu Gly Asp Ile Lys Asp Phe Val Asp Trp Lys Asn 20 25 30	1725
CAA CGC GGT CTC CGT ACC GAG GTG AAA GTG GCA GAA GAT ATT GCT TCT Gln Arg Gly Leu Arg Thr Glu Val Lys Val Ala Glu Asp Ile Ala Ser 35 40 45	1773
CCC GTT ACA GCT AAT GCT ATT CAG CAG TTC GTT AAG CAA GAA TAC GAG Pro Val Thr Ala Asn Ala Ile Gln Gln Phe Val Lys Gln Glu Tyr Glu 50 55 60	1821
AAA GAA GGT AAT GAT TTG ACC TAT GTT CTT TTG GTT GGC GAT CAC AAA Lys Glu Gly Asn Asp Leu Thr Tyr Val Leu Leu Val Gly Asp His Lys 65 70 75 80	1869
GAT ATT CCT GCC AAA ATT ACT CCG GGG ATC AAA TCC GAC CAG GTA TAT Asp Ile Pro Ala Lys Ile Thr Pro Gly Ile Lys Ser Asp Gln Val Tyr 85 90 95	1917
GGA CAA ATA GTA GGT AAT GAC CAC TAC AAC GAA GTC TTC ATC GGT CGT Gly Gln Ile Val Gly Asn Asp His Tyr Asn Glu Val Phe Ile Gly Arg 100 105 110	1965
TTC TCA TGT GAG AGC AAA GAG GAT CTG AAG ACA CAA ATC GAT CGG ACT Phe Ser Cys Glu Ser Lys Glu Asp Leu Lys Thr Gln Ile Asp Arg Thr 115 120 125	2013
ATT CAC TAT GAG CGC AAT ATA ACC ACG GAA GAC AAA TGG CTC GGT CAG Ile His Tyr Glu Arg Asn Ile Thr Thr Glu Asp Lys Trp Leu Gly Gln 130 135 140	2061
GCT CTT TGT ATT GCT TCG GCT GAA GGA GGC CCA TCC GCA GAC AAT GGT Ala Leu Cys Ile Ala Ser Ala Glu Gly Pro Ser Ala Asp Asn Gly 145 150 155 160	2109
GAA AGT GAT ATC CAG CAT GAG AAT GTA ATC GCC AAT CTG CTT ACC CAG Glu Ser Asp Ile Gln His Glu Asn Val Ile Ala Asn Leu Thr Gln 165 170 175	2157

TAT GGC TAT ACC AAG ATT ATC AAA TGT TAT GAT CCG GGA GTA ACT CCT Tyr Gly Tyr Thr Lys Ile Ile Lys Cys Tyr Asp Pro Gly Val Thr Pro 180 185 190	2205
AAA AAC ATT ATT GAT GCT TTC AAC GGA GGA ATC TCG TTG GTC AAC TAT Lys Asn Ile Ile Asp Ala Phe Asn Gly Gly Ile Ser Leu Val Asn Tyr 195 200 205	2253
ACG GGC CAC GGT AGC GAA ACA GCT TGG GGT ACG TCT CAC TTC GGC ACC Thr Gly His Gly Ser Glu Thr Ala Trp Gly Thr Ser His Phe Gly Thr 210 215 220	2301
ACT CAT GTG AAG CAG CTT ACC AAC AGC AAC CAG CTA CCG TTT ATT TTC Thr His Val Lys Gln Leu Thr Asn Ser Asn Gln Leu Pro Phe Ile Phe 225 230 235 240	2349
GAC GTA GCT TGT GTG AAT GGC GAT TTC CTA TTC AGC ATG CCT TGC TTC Asp Val Ala Cys Val Asn Gly Asp Phe Leu Phe Ser Met Pro Cys Phe 245 250 255	2397
GCA GAA GCC CTG ATG CGT GCA CAA AAA GAT GGT AAG CCG ACA GGT ACT Ala Glu Ala Leu Met Arg Ala Gln Lys Asp Gly Lys Pro Thr Gly Thr 260 265 270	2445
GTT GCT ATC ATA GCG TCT ACG ATC AAC CAG TCT TGG GCT TCT CCT ATG Val Ala Ile Ile Ala Ser Thr Ile Asn Gln Ser Trp Ala Ser Pro Met 275 280 285	2493
CGC GGG CAG GAT GAG ATG AAC GAA ATT CTG TGC GAA AAA CAC CCG AAC Arg Gly Gln Asp Glu Met Asn Glu Ile Leu Cys Glu Lys His Pro Asn 290 295 300	2541
AAC ATC AAG CGT ACT TTC GGT GTC ACC ATG AAC GGT ATG TTT GCT Asn Ile Lys Arg Thr Phe Gly Gly Val Thr Met Asn Gly Met Phe Ala 305 310 315 320	2589
ATG GTG GAA AAG TAT AAA AAG GAT GGT GAG AAG ATG CTC GAC ACA TGG Met Val Glu Lys Tyr Lys Asp Gly Glu Lys Met Leu Asp Thr Trp 325 330 335	2637
ACT GTT TTC GGC GAC CCC TCG CTG CTC GTT CGT ACA CTT GTC CCG ACC Thr Val Phe Gly Asp Pro Ser Leu Leu Val Arg Thr Leu Val Pro Thr 340 345 350	2685
AAA ATG CAG GTT ACG GCT CCG GCT CAG ATT AAT TTG ACG GAT GCT TCA Lys Met Gln Val Thr Ala Pro Ala Gln Ile Asn Leu Thr Asp Ala Ser 355 360 365	2733
GTC AAC GTA TCT TGC GAT TAT AAT GGT GCT ATT GCT ACC ATT TCA GCC Val Asn Val Ser Cys Asp Tyr Asn Gly Ala Ile Ala Thr Ile Ser Ala 370 375 380	2781
AAT GGA AAG ATG TTC GGT TCT GCA GTT GTC GAA AAT GGA ACA GCT ACA Asn Gly Lys Met Phe Gly Ser Ala Val Val Glu Asn Gly Thr Ala Thr 385 390 395 400	2829
ATC AAT CTG ACA GGT CTG ACA AAT GAA AGC ACG CTT ACC CTT ACA GTA Ile Asn Leu Thr Gly Leu Thr Asn Glu Ser Thr Leu Thr Leu Thr Val 405 410 415	2877
GTT GGT TAC AAC AAA GAG ACG GTT ATT AAG ACC ATC AAC ACT AAT GGT Val Gly Tyr Asn Lys Glu Thr Val Ile Lys Thr Ile Asn Thr Asn Gly 420 425 430	2925
GAG CCT AAC CCC TAC CAG CCC GTT TCC AAC TTG ACA GCT ACA ACG CAG Glu Pro Asn Pro Tyr Gln Pro Val Ser Asn Leu Thr Ala Thr Thr Gln 435 440 445	2973

GGT CAG AAA GTA ACG CTC AAG TGG GAT GCA CCG AGC ACG AAA ACC AAT Gly Gln Lys Val Thr Leu Lys Trp Asp Ala Pro Ser Thr Lys Thr Asn 450 455 460	3021
GCA ACC ACT AAT ACC GCT CGC AGC GTG GAT GGC ATA CGA GAA TTG GTT Ala Thr Thr Asn Thr Ala Arg Ser Val Asp Gly Ile Arg Glu Leu Val 465 470 475 480	3069
CTT CTG TCA GTC AGC GAT GCC CCC GAA CTT CTT CGC AGC GGT CAG GCC Leu Leu Ser Val Ser Asp Ala Pro Glu Leu Leu Arg Ser Gly Gln Ala 485 490 495	3117
GAG ATT GTT CTT GAA GCT CAC GAT GTT TGG AAT GAT GGA TCC Glu Ile Val Leu Glu Ala His Asp Val Trp Asn Asp Gly Ser 500 505 510	3159

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 737 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Lys Asn Leu Asn Lys Phe Val Ser Ile Ala Leu Cys Ser Ser Leu
 -227 -225 -220 -215

Leu Gly Gly Met Ala Phe Ala Gln Gln Thr Glu Leu Gly Arg Asn Pro
 -210 -205 -200

Asn Val Arg Leu Leu Glu Ser Thr Gln Gln Ser Val Thr Lys Val Gln
 -195 -190 -185 -180

Phe Arg Met Asp Asn Leu Lys Phe Thr Glu Val Gln Thr Pro Lys Gly
 -175 -170 -165

Ile Gly Gln Val Pro Thr Tyr Thr Glu Gly Val Asn Leu Ser Glu Lys
 -160 -155 -150

Gly Met Pro Thr Leu Pro Ile Leu Ser Arg Ser Leu Ala Val Ser Asp
 -145 -140 -135

Thr Arg Glu Met Lys Val Glu Val Val Ser Ser Lys Phe Ile Glu Lys
 -130 -125 -120

Lys Asn Val Leu Ile Ala Pro Ser Lys Gly Met Ile Met Arg Asn Glu
 -115 -110 -105 -100

Asp Pro Lys Lys Ile Pro Tyr Val Tyr Gly Lys Ser Tyr Ser Gln Asn
 -95 -90 -85

Lys Phe Phe Pro Gly Glu Ile Ala Thr Leu Asp Asp Pro Phe Ile Leu
 -80 -75 -70

Arg Asp Val Arg Gly Gln Val Val Asn Phe Ala Pro Leu Gln Tyr Asn
 -65 -60 -55

Pro Val Thr Lys Thr Leu Arg Ile Tyr Thr Glu Ile Thr Val Ala Val
 -50 -45 -40

Ser Glu Thr Ser Glu Gln Gly Lys Asn Ile Leu Asn Lys Lys Gly Thr
 -35 -30 -25 -20

67

Phe Ala Gly Phe Glu Asp Thr Tyr Lys Arg Met Phe Met Asn Tyr Glu
 -15 -10 -5

Pro Gly Arg Tyr Thr Pro Val Glu Glu Lys Gln Asn Gly Arg Met Ile
 1 5 10

Val Ile Val Ala Lys Lys Tyr Glu Gly Asp Ile Lys Asp Phe Val Asp
 15 20 25

Trp Lys Asn Gln Arg Gly Leu Arg Thr Glu Val Lys Val Ala Glu Asp
 30 35 40 45

Ile Ala Ser Pro Val Thr Ala Asn Ala Ile Gln Gln Phe Val Lys Gln
 50 55 60

Glu Tyr Glu Lys Glu Gly Asn Asp Leu Thr Tyr Val Leu Leu Val Gly
 65 70 75

Asp His Lys Asp Ile Pro Ala Lys Ile Thr Pro Gly Ile Lys Ser Asp
 80 85 90

Gln Val Tyr Gly Gln Ile Val Gly Asn Asp His Tyr Asn Glu Val Phe
 95 100 105

Ile Gly Arg Phe Ser Cys Glu Ser Lys Glu Asp Leu Lys Thr Gln Ile
 110 115 120 125

Asp Arg Thr Ile His Tyr Glu Arg Asn Ile Thr Thr Glu Asp Lys Trp
 130 135 140

Leu Gly Gln Ala Leu Cys Ile Ala Ser Ala Glu Gly Gly Pro Ser Ala
 145 150 155

Asp Asn Gly Glu Ser Asp Ile Gln His Glu Asn Val Ile Ala Asn Leu
 160 165 170

Leu Thr Gln Tyr Gly Tyr Thr Lys Ile Ile Lys Cys Tyr Asp Pro Gly
 175 180 185

Val Thr Pro Lys Asn Ile Ile Asp Ala Phe Asn Gly Gly Ile Ser Leu
 190 195 200 205

Val Asn Tyr Thr Gly His Gly Ser Glu Thr Ala Trp Gly Thr Ser His
 210 215 220

Phe Gly Thr Thr His Val Lys Gln Leu Thr Asn Ser Asn Gln Leu Pro
 225 230 235

Phe Ile Phe Asp Val Ala Cys Val Asn Gly Asp Phe Leu Phe Ser Met
 240 245 250

Pro Cys Phe Ala Glu Ala Leu Met Arg Ala Gln Lys Asp Gly Lys Pro
 255 260 265

Thr Gly Thr Val Ala Ile Ile Ala Ser Thr Ile Asn Gln Ser Trp Ala
 270 275 280 285

Ser Pro Met Arg Gly Gln Asp Glu Met Asn Glu Ile Leu Cys Glu Lys
 290 295 300

His Pro Asn Asn Ile Lys Arg Thr Phe Gly Gly Val Thr Met Asn Gly
 305 310 315

Met Phe Ala Met Val Glu Lys Tyr Lys Lys Asp Gly Glu Lys Met Leu
 320 325 330

Asp Thr Trp Thr Val Phe Gly Asp Pro Ser Leu Leu Val Arg Thr Leu
335 340 345

Val Pro Thr Lys Met Gln Val Thr Ala Pro Ala Gln Ile Asn Leu Thr
350 355 360 365

Asp Ala Ser Val Asn Val Ser Cys Asp Tyr Asn Gly Ala Ile Ala Thr
370 375 380

Ile Ser Ala Asn Gly Lys Met Phe Gly Ser Ala Val Val Glu Asn Gly
385 390 395

Thr Ala Thr Ile Asn Leu Thr Gly Leu Thr Asn Glu Ser Thr Leu Thr
400 405 410

Leu Thr Val Val Gly Tyr Asn Lys Glu Thr Val Ile Lys Thr Ile Asn
415 420 425

Thr Asn Gly Glu Pro Asn Pro Tyr Gln Pro Val Ser Asn Leu Thr Ala
430 435 440 445

Thr Thr Gln Gly Gln Lys Val Thr Leu Lys Trp Asp Ala Pro Ser Thr
450 455 460

Lys Thr Asn Ala Thr Thr Asn Thr Ala Arg Ser Val Asp Gly Ile Arg
465 470 475

Glu Leu Val Leu Leu Ser Val Ser Asp Ala Pro Glu Leu Leu Arg Ser
480 485 490

Gly Gln Ala Glu Ile Val Leu Glu Ala His Asp Val Trp Asn Asp Gly
495 500 505

Ser
510

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Glu Leu Leu Arg
1

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

69

(ii) MOLECULE TYPE: DNA (other nucleic acid)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGCTTTACNC CNGTNGARGA RYTNGA

26

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (other nucleic acid)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCTTTRTTY TTCCARTCNA CRAARTCYTT

30

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 48 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (other nucleic acid)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCTGGAGAAT TCTCGTATGA TCGTCATCGT AGCCAAAAAG TATGAGGG

48

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (other nucleic acid)

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATCAACACTA ATGGTGAGCC

20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

70

- (A) LENGTH: 7266 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 949..6063

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTGCAGAGGG CTGGTAAAGA CCGCCTCGGG ATCGAGGCCT TTGAGACGGG CACAAGCCGC	60
CGCAGCCTCC TCTTCGAAGG TGTCTCGAAC GTCCACATCG GTGAATCCGT AGCAGTCCTC	120
ATTGCCATTG AGCAGCACCG AGGTGTGGCG CATCAGATAT ATTTTCATCA GTGGATTATT	180
AGGGTATCGG TCAGAAAAAG CCTTCCGAAT CCGACAAAGA TAGTAGAAAG AGAGTGCATC	240
TGAAAACAGA TCATTGAGG ATTATCGATC AACTGAAAG GCAGGAGTTG TTTTGCCTT	300
TGGTTCGGAA AATTACCTGA TCAGCATTG TAAAAACGTG GCGCGAGAAT TTTTCGTTT	360
TGGCGCGAGA ATTAAAAATT TTTGGAACCA CAGCGAAAAA AATCTCGCCG CGTTTCTCA	420
GGATTTACAG ACCACAATCC GAGCATTTC GGTCGTAAT TCATCGAAGA GACAGGTTT	480
ACCGCATTGA AATCAGAGAG AGAATATCCG TAGTCCAACG GTTCATCCTT ATATCAGAGG	540
TTAAAAGATA TGGTACGCTC ATCGAGGAGC TGATTGGCTT AGTAGGTGAG ACTTTCTTAA	600
GAGACTATCG GCACCTACAG GAAGTTCATG GCACACAAAGG CAAAGGAGGC AATCTCGCA	660
GACCGGACTC ATATCAAAG GATGAAACGA CTTTCCATA CGACAAACCAA ATAGCCGTCT	720
ACGGTAGACG AATGCAAACC CAATATGAGG CCATCAATCA ATCCGAATGA CAGCTTTGG	780
GCAATATATT ATGCATATT TGATTGCGT TTAAAGGAAA AGTGCATATA TTTGCGATTG	840
TGGTATTCTT TTGGTTCT ATGTGAATT TGCTCCCAA GAAGACTTTA TAATGCATAA	900
ATACAGAAGG GGTACTACAC AGTAAAATCA TATTCTAATT TCATCAAA ATG AAA AAC	957
Met Lys Asn	
1	
TTG AAC AAG TTT GTT TCG ATT GCT CTT TGC TCT TCC TTA TTA GGA GGA	1005
Leu Asn Lys Phe Val Ser Ile Ala Leu Cys Ser Ser Leu Leu Gly Gly	
5 10 15	
ATG GCA TTT GCG CAG CAG ACA GAG TTG GGA CGC AAT CCG AAT GTC AGA	1053
Met Ala Phe Ala Gln Gln Thr Glu Leu Gly Arg Asn Pro Asn Val Arg	
20 25 30 35	
TTG CTC GAA TCC ACT CAG CAA TCG GTG ACA AAG GTT CAG TTC CGT ATG	1101
Leu Leu Glu Ser Thr Gln Gln Ser Val Thr Lys Val Gln Phe Arg Met	
40 45 50	

GAC AAC CTC AAG TTC ACC GAA GTT CAA ACC CCT AAG GGA ATC GGA CAA Asp Asn Leu Lys Phe Thr Glu Val Gln Thr Pro Lys Gly Ile Gly Gln 55 60 65	1149
GTG CCG ACC TAT ACA GAA GGG GTT AAT CTT TCC GAA AAA GGG ATG CCT Val Pro Thr Tyr Thr Glu Gly Val Asn Leu Ser Glu Lys Gly Met Pro 70 75 80	1197
ACG CTT CCC ATT CTA TCA CGC TCT TTG GCG GTT TCA GAC ACT CGT GAG Thr Leu Pro Ile Leu Ser Arg Ser Leu Ala Val Ser Asp Thr Arg Glu 85 90 95	1245
ATG AAG GTA GAG GTT TCC TCA AAG TTC ATC GAA AAG AAA AAT GTC Met Lys Val Glu Val Val Ser Ser Lys Phe Ile Glu Lys Lys Asn Val 100 105 110 115	1293
CTG ATT GCA CCC TCC AAG GGC ATG ATT ATG CGT AAC GAA GAT CCG AAA Leu Ile Ala Pro Ser Lys Gly Met Ile Met Arg Asn Glu Asp Pro Lys 120 125 130	1341
AAG ATC CCT TAC GTT TAT GGA AAG AGC TAC TCG CAA AAC AAA TTC TTC Lys Ile Pro Tyr Val Tyr Gly Lys Ser Tyr Ser Gln Asn Lys Phe Phe 135 140 145	1389
CCG GGA GAG ATC GCC ACG CTT GAT GAT CCT TTT ATC CTT CGT GAT GTG Pro Gly Glu Ile Ala Thr Leu Asp Asp Pro Phe Ile Leu Arg Asp Val 150 155 160	1437
CGT GGA CAG GTT GTA AAC TTT GCG CCT TTG CAG TAT AAC CCT GTG ACA Arg Gly Gln Val Val Asn Phe Ala Pro Leu Gln Tyr Asn Pro Val Thr 165 170 175	1485
AAG ACG TTG CGC ATC TAT ACG GAA ATC ACT GTG GCA GTG AGC GAA ACT Lys Thr Leu Arg Ile Tyr Thr Glu Ile Thr Val Ala Val Ser Glu Thr 180 185 190 195	1533
TCG GAA CAA GGC AAA AAT ATT CTG AAC AAG AAA GGT ACA TTT GCC GGC Ser Glu Gln Gly Lys Asn Ile Leu Asn Lys Lys Gly Thr Phe Ala Gly 200 205 210	1581
TTT GAA GAC ACA TAC AAG CGC ATG TTC ATG AAC TAC GAG CCG GGG CGT Phe Glu Asp Thr Tyr Lys Arg Met Phe Met Asn Tyr Glu Pro Gly Arg 215 220 225	1629
TAC ACA CCG GTA GAG GAA AAA CAA AAT GGT CGT ATG ATC GTC ATC GTA Tyr Thr Pro Val Glu Glu Lys Gln Asn Gly Arg Met Ile Val Ile Val 230 235 240	1677
GCC AAA AAG TAT GAG GGA GAT ATT AAA GAT TTC GTT GAT TGG AAA AAC Ala Lys Tyr Glu Gly Asp Ile Lys Asp Phe Val Asp Trp Lys Asn 245 250 255	1725
CAA CGC GGT CTC CGT ACC GAG GTG AAA GTG CCA GAA GAT ATT GCT TCT Gln Arg Gly Leu Arg Thr Glu Val Lys Val Ala Glu Asp Ile Ala Ser 260 265 270 275	1773
CCC GTT ACA GCT AAT GCT ATT CAG CAG TTC GTT AAG CAA GAA TAC GAG Pro Val Thr Ala Asn Ala Ile Gln Gln Phe Val Lys Glu Tyr Glu 280 285 290	1821
AAA GAA GGT AAT GAT TTG ACC TAT GTT CTT TTG GTT GGC GAT CAC AAA Lys Glu Gly Asn Asp Leu Thr Tyr Val Leu Leu Val Gly Asp His Lys 295 300 305	1869

GAT ATT CCT GCC AAA ATT ACT CCG GGG ATC AAA TCC GAC CAG GTA TAT Asp Ile Pro Ala Lys Ile Thr Pro Gly Ile Lys Ser Asp Gln Val Tyr 310 315 320	1917
GGA CAA ATA GTA GGT AAT GAC CAC TAC AAC GAA GTC TTC ATC GGT CGT Gly Gln Ile Val Gly Asn Asp His Tyr Asn Glu Val Phe Ile Gly Arg 325 330 335	1965
TTC TCA TGT GAG AGC AAA GAG GAT CTG AAG ACA CAA ATC GAT CGG ACT Phe Ser Cys Glu Ser Lys Glu Asp Leu Lys Thr Gln Ile Asp Arg Thr 340 345 350 355	2013
ATT CAC TAT GAG CGC AAT ATA ACC ACG GAA GAC AAA TGG CTC GGT CAG Ile His Tyr Glu Arg Asn Ile Thr Thr Glu Asp Lys Trp Leu Gly Gln 360 365 370	2061
GCT CTT TGT ATT GCT TCG GCT GAA GGA GGC CCA TCC GCA GAC AAT GGT Ala Leu Cys Ile Ala Ser Ala Glu Gly Gly Pro Ser Ala Asp Asn Gly 375 380 385	2109
GAA AGT GAT ATC CAG CAT GAG AAT GTA ATC GCC AAT CTG CTT ACC CAG Glu Ser Asp Ile Gln His Glu Asn Val Ile Ala Asn Leu Leu Thr Gln 390 395 400	2157
TAT GGC TAT ACC AAG ATT ATC AAA TGT TAT GAT CCG GGA GTA ACT CCT Tyr Gly Tyr Thr Lys Ile Ile Lys Cys Tyr Asp Pro Gly Val Thr Pro 405 410 415	2205
AAA AAC ATT ATT GAT GCT TTC AAC GGA GGA ATC TCG TTG GTC AAC TAT Lys Asn Ile Ile Asp Ala Phe Asn Gly Gly Ile Ser Leu Val Asn Tyr 420 425 430 435	2253
ACG GGC CAC GGT AGC GAA ACA GCT TGG GGT ACG TCT CAC TTC GGC ACC Thr Gly His Gly Ser Glu Thr Ala Trp Gly Thr Ser His Phe Gly Thr 440 445 450	2301
ACT CAT GTG AAG CAG CTT ACC AAC AGC AAC CAG CTA CCG TTT ATT TTC Thr His Val Lys Gln Leu Thr Asn Ser Asn Gln Leu Pro Phe Ile Phe 455 460 465	2349
GAC GTA GCT TGT GTG AAT GGC GAT TTC CTA TTC AGC ATG CCT TGC TTC Asp Val Ala Cys Val Asn Gly Asp Phe Leu Phe Ser Met Pro Cys Phe 470 475 480	2397
GCA GAA GCC CTG ATG CGT GCA CAA AAA GAT GGT AAG CCG ACA GGT ACT Ala Glu Ala Leu Met Arg Ala Gln Lys Asp Gly Lys Pro Thr Gly Thr 485 490 495	2445
GTT GCT ATC ATA GCG TCT ACG ATC AAC CAG TCT TGG GCT TCT CCT ATG Val Ala Ile Ile Ala Ser Thr Ile Asn Gln Ser Trp Ala Ser Pro Met 500 505 510 515	2493
CGC GGG CAG GAT GAG ATG AAC GAA ATT CTG TGC GAA AAA CAC CCG AAC Arg Gly Gln Asp Glu Met Asn Glu Ile Leu Cys Glu Lys His Pro Asn 520 525 530	2541
AAC ATC AAG CGT ACT TTC GGT GTC ACC ATG AAC GGT ATG TTT GCT Asn Ile Lys Arg Thr Phe Gly Gly Val Thr Met Asn Gly Met Phe Ala 535 540 545	2589
ATG GTG GAA AAG TAT AAA AAG GAT GGT GAG AAG ATG CTC GAC ACA TGG Met Val Glu Lys Tyr Lys Lys Asp Gly Glu Lys Met Leu Asp Thr Trp 550 555 560	2637

ACT GTT TTC GGC GAC CCC TCG CTG CTC GTT CGT ACA CTT GTC CCG ACC Thr Val Phe Gly Asp Pro Ser Leu Leu Val Arg Thr Leu Val Pro Thr 565 570 575	2685
AAA ATG CAG GTT ACG GCT CCG GCT CAG ATT AAT TTG ACG GAT GCT TCA Lys Met Gln Val Thr Ala Pro Ala Gln Ile Asn Leu Thr Asp Ala Ser 580 585 590 595	2733
GTC AAC GTA TCT TGC GAT TAT AAT GGT GCT ATT GCT ACC ATT TCA GCC Val Asn Val Ser Cys Asp Tyr Asn Gly Ala Ile Ala Thr Ile Ser Ala 600 605 610	2781
AAT GGA AAG ATG TTC GGT TCT GCA GTT GTC GAA AAT GGA ACA GCT ACA Asn Gly Lys Met Phe Gly Ser Ala Val Val Glu Asn Gly Thr Ala Thr 615 620 625	2829
ATC AAT CTG ACA GGT CTG ACA AAT GAA AGC ACG CTT ACC CTT ACA GTA Ile Asn Leu Thr Gly Leu Thr Asn Glu Ser Thr Leu Thr Leu Thr Val 630 635 640	2877
GTT GGT TAC AAC AAA GAG ACG GTT ATT AAG ACC ATC AAC ACT AAT GGT Val Gly Tyr Asn Lys Glu Thr Val Ile Lys Thr Ile Asn Thr Asn Gly 645 650 655	2925
GAG CCT AAC CCC TAC CAG CCC GTT TCC AAC TTG ACA GCT ACA ACG CAG Glu Pro Asn Pro Tyr Gln Pro Val Ser Asn Leu Thr Ala Thr Thr Gln 660 665 670 675	2973
GGT CAG AAA GTA ACG CTC AAG TGG GAT GCA CCG AGC ACG AAA ACC AAT Gly Gln Lys Val Thr Leu Lys Trp Asp Ala Pro Ser Thr Lys Thr Asn 680 685 690	3021
GCA ACC ACT AAT ACC GCT CGC AGC GTG GAT GGC ATA CGA GAA TTG GTT Ala Thr Thr Asn Thr Ala Arg Ser Val Asp Gly Ile Arg Glu Leu Val 695 700 705	3069
CTT CTG TCA GTC AGC GAT GCC CCC GAA CTT CTT CGC AGC GGT CAG GCC Leu Leu Ser Val Ser Asp Ala Pro Glu Leu Leu Arg Ser Gly Gln Ala 710 715 720	3117
GAG ATT GTT CTT GAA GCT CAC GAT GTT TGG AAT GAT GGA TCC GGT TAT Glu Ile Val Leu Glu Ala His Asp Val Trp Asn Asp Gly Ser Gly Tyr 725 730 735	3165
CAG ATT CTT TTG GAT GCA GAC CAT GAT CAA TAT GGA CAG GTT ATA CCC Gln Ile Leu Leu Asp Ala Asp His Asp Gln Tyr Gly Gln Val Ile Pro 740 745 750 755	3213
AGT GAT ACC CAT ACT CTT TGG CCG AAC TGT AGT GTC CCG GCC AAT CTG Ser Asp Thr His Thr Leu Trp Pro Asn Cys Ser Val Pro Ala Asn Leu 760 765 770	3261
TTC GCT CCG TTC GAA TAT ACT GTT CCG GAA AAT GCA GAT CCT TCT TGT Phe Ala Pro Phe Glu Tyr Thr Val Pro Glu Asn Ala Asp Pro Ser Cys 775 780 785	3309
TCC CCT ACC AAT ATG ATA ATG GAT GGT ACT GCA TCC GTT AAT ATA CCG Ser Pro Thr Asn Met Ile Met Asp Gly Thr Ala Ser Val Asn Ile Pro 790 795 800	3357
GCC GGA ACT TAT GAC TTT GCA ATT GCT GCT CCT CAA GCA AAT GCA AAG Ala Gly Thr Tyr Asp Phe Ala Ile Ala Ala Pro Gln Ala Asn Ala Lys 805 810 815	3405
ATT TGG ATT GCC GGA CAA GGA CCG ACG AAA GAA GAT GAT TAT GTA TTT Ile Trp Ile Ala Gly Gln Gly Pro Thr Lys Glu Asp Asp Tyr Val Phe 820 825 830 835	3453

GAA	GCC	GGT	AAA	AAA	TAC	CAT	TTC	CTT	ATG	AAG	AAG	ATG	GGT	AGC	GGT		3501
Glu	Ala	Gly	Lys	Lys	Tyr	His	Phe	Leu	Met	Lys	Lys	Met	Gly	Ser	Gly		
840									845							850	
GAT	GGA	ACT	GAA	TTG	ACT	ATA	AGC	GAA	GGT	GGT	GGA	AGC	GAT	TAC	ACC		3549
Asp	Gly	Thr	Glu	Leu	Thr	Ile	Ser	Glu	Gly	Gly	Gly	Ser	Asp	Tyr	Thr		
855								860								865	
TAT	ACT	GTC	TAT	CGT	GAC	GGC	ACG	AAG	ATC	AAG	GAA	GGT	CTG	ACG	GCT		3597
Tyr	Thr	Val	Tyr	Arg	Asp	Gly	Thr	Lys	Ile	Lys	Glu	Gly	Leu	Thr	Ala		
870								875								880	
ACG	ACA	TTC	GAA	GAA	GAC	GGT	GTA	GCT	ACG	GGC	AAT	CAT	GAG	TAT	TGC		3645
Thr	Thr	Phe	Glu	Glu	Asp	Gly	Val	Ala	Thr	Gly	Asn	His	Glu	Tyr	Cys		
885							890				895						
GTG	GAA	GTT	AAG	TAC	ACA	GCC	GGC	GTA	TCT	CCG	AAG	GTA	TGT	AAA	GAC		3693
Val	Glu	Val	Lys	Tyr	Thr	Ala	Gly	Val	Ser	Pro	Lys	Val	Cys	Lys	Asp		
900							905			910					915		
GTT	ACG	GTA	GAA	GGA	TCC	AAT	GAA	TTT	GCT	CCT	GTA	CAG	AAC	CTG	ACC		3741
Val	Thr	Val	Glu	Gly	Ser	Asn	Glu	Phe	Ala	Pro	Val	Gln	Asn	Leu	Thr		
920							925								930		
GGT	AGT	GCA	GTC	GGC	CAG	AAA	GTA	ACG	CTC	AAG	TGG	GAT	GCA	CCT	AAT		3789
Gly	Ser	Ala	Val	Gly	Gln	Lys	Val	Thr	Leu	Lys	Trp	Asp	Ala	Pro	Asn		
935							940								945		
GGT	ACC	CCG	AAT	CCA	AAT	CCG	AAT	CCG	AAT	CCC	GGA	ACA	ACA			3837	
Gly	Thr	Pro	Asn	Pro	Asn	Pro	Asn	Pro	Asn	Pro	Gly	Thr	Thr				
950							955			960							
ACA	CTT	TCC	GAA	TCA	TTC	GAA	AAT	GGT	ATT	CCT	GCC	TCA	TGG	AAG	ACG		3885
Thr	Leu	Ser	Glu	Ser	Phe	Glu	Asn	Gly	Ile	Pro	Ala	Ser	Trp	Lys	Thr		
965							970			975							
ATC	GAT	GCA	GAC	GGT	GAC	GGG	CAT	GGC	TGG	AAG	CCT	GGA	AAT	GCT	CCC		3933
Ile	Asp	Ala	Asp	Gly	Asp	Gly	His	Gly	Trp	Lys	Pro	Gly	Asn	Ala	Pro		
980							985			990					995		
GGA	ATC	GCT	GGC	TAC	AAT	AGC	AAT	GGT	TGT	GTA	TAT	TCA	GAG	TCA	TTC		3981
Gly	Ile	Ala	Gly	Tyr	Asn	Ser	Asn	Gly	Cys	Val	Tyr	Ser	Glu	Ser	Phe		
1000							1005			1010							
GGT	CTT	GGT	GGT	ATA	GGA	GTT	CTT	ACC	CCT	GAC	AAC	TAT	CTG	ATA	ACA		4029
Gly	Leu	Gly	Ile	Gly	Val	Leu	Thr	Pro	Asp	Asn	Tyr	Leu	Ile	Thr			
1015							1020			1025							
CCG	GCA	TTG	GAT	TTG	CCT	AAC	GGA	GGT	AAG	TTG	ACT	TTC	TGG	GTA	TGC		4077
Pro	Ala	Leu	Asp	Leu	Pro	Asn	Gly	Gly	Lys	Leu	Thr	Phe	Trp	Val	Cys		
1030							1035			1040							
GCA	CAG	GAT	GCT	AAT	TAT	GCA	TCC	GAG	CAC	TAT	GCG	GTG	TAT	GCA	TCT		4125
Ala	Gln	Asp	Ala	Asn	Tyr	Ala	Ser	Glu	His	Tyr	Ala	Val	Tyr	Ala	Ser		
1045							1050			1055							
TCG	ACC	GGT	AAC	GAT	GCA	TCC	AAC	TTC	ACG	AAT	GCT	TTG	TTG	GAA	GAG		4173
Ser	Thr	Gly	Asn	Asp	Ala	Ser	Asn	Phe	Thr	Asn	Ala	Leu	Leu	Glu	Glu		
1060							1065			1070					1075		
ACG	ATT	ACG	GCA	AAA	GGT	GTT	CGC	TCG	CCG	GAA	GCT	ATT	CGT	GGT	CGT		4221
Thr	Ile	Thr	Ala	Lys	Gly	Val	Arg	Ser	Pro	Glu	Ala	Ile	Arg	Gly	Arg		
1080							1085			1090							
ATA	CAG	GGT	ACT	TGG	CGC	CAG	AAG	ACG	GTA	GAC	CTT	CCC	GCA	GGT	ACG		4269
Ile	Gln	Gly	Thr	Trp	Arg	Gln	Lys	Thr	Val	Asp	Leu	Pro	Ala	Gly	Thr		
1095							1100			1105							

AAA TAT GTT GCT TTC CGT CAC TTC CAA AGC ACG GAT ATG TTC TAC ATC Lys Tyr Val Ala Phe Arg His Phe Gln Ser Thr Asp Met Phe Tyr Ile 1110 1115 1120	4317
GAC CTT GAT GAG GTT GAG ATC AAG GCC AAC GCC AAG CGC GCA GAC TTC Asp Leu Asp Glu Val Glu Ile Lys Ala Asn Gly Lys Arg Ala Asp Phe 1125 1130 1135	4365
ACG GAA ACG TTC GAG TCT TCT ACT CAT GGA GAG GCA CCG GCG GAA TGG Thr Glu Thr Phe Glu Ser Ser Thr His Gly Glu Ala Pro Ala Glu Trp 1140 1145 1150 1155	4413
ACT ACT ATC GAT GCC GAT GGC GAT GGT CAG GGT TGG CTC TGT CTG TCT Thr Thr Ile Asp Ala Asp Gly Asp Gly Gln Gly Trp Leu Cys Leu Ser 1160 1165 1170	4461
TCC GGA CAA TTG GAC TGG CTG ACA GCT CAT GGC GGC ACC AAC GTA GTA Ser Gly Gln Leu Asp Trp Leu Thr Ala His Gly Gly Thr Asn Val Val 1175 1180 1185	4509
GCC TCT TTC TCA TGG AAT GGA ATG GCT TTG AAT CCT GAT AAC TAT CTC Ala Ser Phe Ser Trp Asn Gly Met Ala Leu Asn Pro Asp Asn Tyr Leu 1190 1195 1200	4557
ATC TCA AAG GAT GTT ACA GGC GCA ACG AAG GTA AAG TAC TAC TAT GCA Ile Ser Lys Asp Val Thr Gly Ala Thr Lys Val Lys Tyr Tyr Tyr Ala 1205 1210 1215	4605
GTC AAC GAC GGT TTT CCC GGG GAT CAC TAT GCG GTG ATG ATC TCC AAG Val Asn Asp Gly Phe Pro Gly Asp His Tyr Ala Val Met Ile Ser Lys 1220 1225 1230 1235	4653
ACG GGC ACG AAC GCC GGA GAC TTC ACG GTT GTT TTC GAA GAA ACG CCT Thr Gly Thr Asn Ala Gly Asp Phe Thr Val Val Phe Glu Glu Thr Pro 1240 1245 1250	4701
AAC GGA ATA AAT AAG GGC GGA GCA AGA TTC GGT CTT TCC ACG GAA GCC Asn Gly Ile Asn Lys Gly Gly Ala Arg Phe Gly Leu Ser Thr Glu Ala 1255 1260 1265	4749
AAT GCC GCC AAA CCT CAA AGT GTA TGG ATC GAG CGT ACG GTA GAT TTG Asn Gly Ala Lys Pro Gln Ser Val Trp Ile Glu Arg Thr Val Asp Leu 1270 1275 1280	4797
CCT GCG GGC ACG AAG TAT GTT GCT TTC CGT CAC TAC AAT TGC TCG GAT Pro Ala Gly Thr Lys Tyr Val Ala Phe Arg His Tyr Asn Cys Ser Asp 1285 1290 1295	4845
TTG AAC TAC ATT CTT TTG GAT ATT CAG TTC ACC ATG GGT GGC AGC Leu Asn Tyr Ile Leu Leu Asp Asp Ile Gln Phe Thr Met Gly Gly Ser 1300 1305 1310 1315	4893
CCC ACC CCG ACC GAT TAT ACC TAC ACG GTG TAT CGT GAC GGT ACG AAG Pro Thr Pro Thr Asp Tyr Thr Tyr Val Tyr Arg Asp Gly Thr Lys 1320 1325 1330	4941
ATC AAG GAA GGT CTG ACC GAA ACG ACC TTC GAA GAA GAC GGC GTA GCT Ile Lys Glu Gly Leu Thr Glu Thr Thr Phe Glu Glu Asp Gly Val Ala 1335 1340 1345	4989
ACA GCC AAT CAT GAG TAT TGC GTG GAA GTG AAG TAC ACA GCC GGC GTA Thr Gly Asn His Glu Tyr Cys Val Glu Val Lys Tyr Thr Ala Gly Val 1350 1355 1360	5037
TCT CCG AAA GAG TGC GTA AAC GTA ACT ATT AAT CCG ACT CAG TTC AAT Ser Pro Lys Glu Cys Val Asn Val Thr Ile Asn Pro Thr Gln Phe Asn 1365 1370 1375	5085

76.

CCT GTA AAG AAC CTG AAG GCA CAA CCG GAT GGC GGC GAC GTG GTT CTC Pro Val Lys Asn Leu Lys Ala Gln Pro Asp Gly Gly Asp Val Val Leu 1380 1385 1390 1395	5133
AAG TGG GAA GCC CCG AGC GCA AAA AAG ACA GAA GGT TCT CGT GAA GTA Lys Trp Glu Ala Pro Ser Ala Lys Lys Thr Glu Gly Ser Arg Glu Val 1400 1405 1410	5181
AAA CGG ATC GGA GAC GGT CTT TTC GTT ACG ATC GAA CCT GCA AAC GAT Lys Arg Ile Gly Asp Gly Leu Phe Val Thr Ile Glu Pro Ala Asn Asp 1415 1420 1425	5229
GTA CGT GCC AAC GAA GCC AAG GTT GTG CTC GCA GCA GAC AAC GTA TGG Val Arg Ala Asn Glu Ala Lys Val Val Leu Ala Ala Asp Asn Val Trp 1430 1435 1440	5277
GGA GAC AAT ACG GGT TAC CAG TTC TTG TTG GAT GCC GAT CAC AAT ACA Gly Asp Asn Thr Gly Tyr Gln Phe Leu Leu Asp Ala Asp His Asn Thr 1445 1450 1455	5325
TTC GGA AGT GTC ATT CCG GCA ACC GGT CCT CTC TTT ACC GGA ACA GCT Phe Gly Ser Val Ile Pro Ala Thr Gly Pro Leu Phe Thr Gly Thr Ala 1460 1465 1470 1475	5373
TCT TCC AAT CTT TAC AGT GCG AAC TTC GAG TAT TTG ATC CCG GCC AAT Ser Ser Asn Leu Tyr Ser Ala Asn Phe Glu Tyr Leu Ile Pro Ala Asn 1480 1485 1490	5421
GCC GAT CCT GTT GTT ACT ACA CAG AAT ATT ATC GTT ACA GGA CAG GGT Ala Asp Pro Val Val Thr Thr Gln Asn Ile Ile Val Thr Gly Gln Gly 1495 1500 1505	5469
GAA GTT GTA ATC CCC GGT GGT GTT TAC GAC TAT TGC ATT ACG AAC CCG Glu Val Val Ile Pro Gly Gly Val Tyr Asp Tyr Cys Ile Thr Asn Pro 1510 1515 1520	5517
GAA CCT GCA TCC GGA AAG ATG TGG ATC GCA GGA GAT GGA GGC AAC CAG Glu Pro Ala Ser Gly Lys Met Trp Ile Ala Gly Asp Gly Gly Asn Gln 1525 1530 1535	5565
CCT GCA CGT TAT GAC GAT TTC ACA TTC GAA GCA GGC AAG AAG TAC ACC Pro Ala Arg Tyr Asp Asp Phe Thr Phe Glu Ala Gly Lys Lys Tyr Thr 1540 1545 1550 1555	5613
TTC ACG ATG CGT CGC GCC GGA ATG GGA GAT GGA ACT GAT ATG GAA GTC Phe Thr Met Arg Arg Ala Gly Met Gly Asp Gly Thr Asp Met Glu Val 1560 1565 1570	5661
GAA GAC GAT TCA CCT GCA AGC TAT ACC TAT ACA GTC TAT CGT GAC GGC Glu Asp Asp Ser Pro Ala Ser Tyr Thr Tyr Thr Val Tyr Arg Asp Gly 1575 1580 1585	5709
ACG AAG ATC AAG GAA GGT CTG ACC GAA ACG ACC TAC CGC GAT GCA GGA Thr Lys Ile Lys Glu Gly Leu Thr Glu Thr Thr Tyr Arg Asp Ala Gly 1590 1595 1600	5757
ATG AGT GCA CAA TCT CAT GAG TAT TGC GTA GAG GTT AAG TAC GCA GCC Met Ser Ala Gln Ser His Glu Tyr Cys Val Glu Val Lys Tyr Ala Ala 1605 1610 1615	5805
GGC GTA TCT CCG AAG GTT TGT GTG GAT TAT ATT CCT GAC GGA GTG GCA Gly Val Ser Pro Lys Val Cys Val Asp Tyr Ile Pro Asp Gly Val Ala 1620 1625 1630 1635	5853
GAC GTA ACG GCT CAG AAG CCT TAC ACC CTG ACA GTT GTT GGA AAG ACG Asp Val Thr Ala Gln Lys Pro Tyr Thr Leu Thr Val Val Gly Lys Thr 1640 1645 1650	5901

ATC ACG GTA ACT TGC CAA GGC GAA GCT ATG ATC TAC GAC ATG AAC GGT Ile Thr Val Thr Cys Gln Gly Glu Ala Met Ile Tyr Asp Met Asn Gly 1655 1660 1665	5949
CGT CGT CTG CCA GCC GGT CGC AAC ACA GTT GTT TAC ACG GCT CAG GGC Arg Arg Leu Ala Ala Gly Arg Asn Thr Val Val Tyr Thr Ala Gln Gly 1670 1675 1680	5997
GGC TAC TAT GCA GTC ATG GTT GTC GTT GAC GGC AAG TCT TAC GTA GAG Gly Tyr Tyr Ala Val Met Val Val Val Asp Gly Lys Ser Tyr Val Glu 1685 1690 1695	6045
AAA CTC GCT GTA AAG TAATTCTGTC TTGGACTCGG AGACTTTGTG CAGACACTTT Lys Leu Ala Val Lys 1700 170	6100
TAATATAGGT CTGTAATTGT CTCAGAGTAT GAATCGATCG CCCGACCTCC TTTTAAGGAA GTCTGGCGA CTTCGTTTT ATGCCTATTA TTCTAATATA CTTCTGAAAC AATTGTTCC	6160
AAAAAGTTGC ATGAAAAGAT TATCTTACTA TCTTGCACT GCAAAAGGGG AGTTTCTAA	6220
GGTTTCCCC GGAGTAGTAC GGTAATAACG GTGTGGTAGT TCAGCTGGTT AGAATACCTG	6280
CCTGTCACGC AGGGGGTCGC GGGTTGAGT CCCGTCCATA CCGCTAAATA GCTGAAAGAT	6340
AGGCTATAGG TCATCTGAAG CAATTTAGA AACGAATCCA AAAGCGTCTT AATTCCAACG	6400
AATTAAGGCG CTTTTCTTT GTCGCCACCC CACACGTCGG ATGAGGTTCG GAATAGGCGT	6460
ATATTCCGTA AATATGCCCTC CGGTGGTTCC ATTTGGTTA CAAAAAACAA AGGGGCTGAA	6520
AATTGTAACC ACAGACGACG TTAAGACGAT GTTTAGACGA TTGACAAATT ACTCTGTTTC	6580
AAAATCATAT GTCGAACTTT GTAGCCGTAT GGTTACACTA ATTTGGAGC AAAATGAAGA	6640
GTCAATTTCG TTCAGTTTT TACTTGCAGCA GCAATTACAT CAACAAAGAA GGTAAAACTC	6700
CTGTCCTTAT TCGTATTTAT CTGAAATAAGG AACGCCGTGC GTTGGGTTCG ACAGGGCTGG	6760
CTGTTAATCC CATAACAATGG GATTCAAGAAA AAGAGAAAGT CAAAGGACAT AGTGCAGAAG	6820
CACTTGAAGT CAATCGAAAG ATCGAAGAAA TCAGGGCTGA TATTCTGACC ATTTACAAAC	6880
GTTTGGAAAGT AACAGTAGAT GATTTGACGC CGGAGAGGAT CAAATCGGAA TACTGCGGAC	6940
AGACGGATAC ATTAAACAGT ATAGTGGAAC TTTTCGATAA ACATAACGAG GATGTCCGGG	7000
CCCAGGTGGG AATCAATAAA ACGGCTGCCA CTTTACAAA ATACGAAAC AGCAAACGGC	7060
ATTTTACCCG ATTCCCTAAA GCGAAGTACA ACAGAACGGA TCTCAAATTC TCAGAGCTTA	7120
CCCCGTTGGT CATTCTAAAC TTTGAGATAT ATCTGCTGAC TGTAGCCCAT TGTTGCCGA	7180
ATACGGCAAC CAAAATCTTC AAGCTT	7240
	7266

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1704 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Lys Asn Leu Asn Lys Phe Val Ser Ile Ala Leu Cys Ser Ser Leu
1 5 10 15

Leu Gly Gly Met Ala Phe Ala Gln Gln Thr Glu Leu Gly Arg Asn Pro
20 25 30

Asn Val Arg Leu Leu Glu Ser Thr Gln Gln Ser Val Thr Lys Val Gln
35 40 45

Phe Arg Met Asp Asn Leu Lys Phe Thr Glu Val Gln Thr Pro Lys Gly
50 55 60

Ile Gly Gln Val Pro Thr Tyr Thr Glu Gly Val Asn Leu Ser Glu Lys
65 70 75 80

Gly Met Pro Thr Leu Pro Ile Leu Ser Arg Ser Leu Ala Val Ser Asp
85 90 95

Thr Arg Glu Met Lys Val Glu Val Val Ser Ser Lys Phe Ile Glu Lys
100 105 110

Lys Asn Val Leu Ile Ala Pro Ser Lys Gly Met Ile Met Arg Asn Glu
115 120 125

Asp Pro Lys Lys Ile Pro Tyr Val Tyr Gly Lys Ser Tyr Ser Gln Asn
130 135 140

Lys Phe Phe Pro Gly Glu Ile Ala Thr Leu Asp Asp Pro Phe Ile Leu
145 150 155 160

Arg Asp Val Arg Gly Gln Val Val Asn Phe Ala Pro Leu Gln Tyr Asn
165 170 175

Pro Val Thr Lys Thr Leu Arg Ile Tyr Thr Glu Ile Thr Val Ala Val
180 185 190

Ser Glu Thr Ser Glu Gln Gly Lys Asn Ile Leu Asn Lys Lys Gly Thr
195 200 205

Phe Ala Gly Phe Glu Asp Thr Tyr Lys Arg Met Phe Met Asn Tyr Glu
210 215 220

Pro Gly Arg Tyr Thr Pro Val Glu Glu Lys Gln Asn Gly Arg Met Ile
225 230 235 240

Val Ile Val Ala Lys Lys Tyr Glu Gly Asp Ile Lys Asp Phe Val Asp
245 250 255

Trp Lys Asn Gln Arg Gly Leu Arg Thr Glu Val Lys Val Ala Glu Asp
260 265 270

Ile Ala Ser Pro Val Thr Ala Asn Ala Ile Gln Gln Phe Val Lys Gln
275 280 285

Glu Tyr Glu Lys Glu Gly Asn Asp Leu Thr Tyr Val Leu Leu Val Gly
290 295 300

Asp His Lys Asp Ile Pro Ala Lys Ile Thr Pro Gly Ile Lys Ser Asp
305 310 315 320

Gln Val Tyr Gly Gln Ile Val Gly Asn Asp His Tyr Asn Glu Val Phe
325 330 335

Ile Gly Arg Phe Ser Cys Glu Ser Lys Glu Asp Leu Lys Thr Gln Ile
340 345 350

Asp Arg Thr Ile His Tyr Glu Arg Asn Ile Thr Thr Glu Asp Lys Trp
355 360 365

Leu Gly Gln Ala Leu Cys Ile Ala Ser Ala Glu Gly Gly Pro Ser Ala
370 375 380

Asp Asn Gly Glu Ser Asp Ile Gln His Glu Asn Val Ile Ala Asn Leu
385 390 395 400

Leu Thr Gln Tyr Gly Tyr Thr Lys Ile Ile Lys Cys Tyr Asp Pro Gly
405 410 415

Val Thr Pro Lys Asn Ile Ile Asp Ala Phe Asn Gly Gly Ile Ser Leu
420 425 430

Val Asn Tyr Thr Gly His Gly Ser Glu Thr Ala Trp Gly Thr Ser His
435 440 445

Phe Gly Thr Thr His Val Lys Gln Leu Thr Asn Ser Asn Gln Leu Pro
450 455 460

Phe Ile Phe Asp Val Ala Cys Val Asn Gly Asp Phe Leu Phe Ser Met
465 470 475 480

Pro Cys Phe Ala Glu Ala Leu Met Arg Ala Gln Lys Asp Gly Lys Pro
485 490 495

Thr Gly Thr Val Ala Ile Ile Ala Ser Thr Ile Asn Gln Ser Trp Ala
500 505 510

Ser Pro Met Arg Gly Gln Asp Glu Met Asn Glu Ile Leu Cys Glu Lys
515 520 525

His Pro Asn Asn Ile Lys Arg Thr Phe Gly Gly Val Thr Met Asn Gly
530 535 540

Met Phe Ala Met Val Glu Lys Tyr Lys Lys Asp Gly Glu Lys Met Leu
545 550 555 560

Asp Thr Trp Thr Val Phe Gly Asp Pro Ser Leu Leu Val Arg Thr Leu
565 570 575

Val Pro Thr Lys Met Gln Val Thr Ala Pro Ala Gln Ile Asn Leu Thr
580 585 590

Asp Ala Ser Val Asn Val Ser Cys Asp Tyr Asn Gly Ala Ile Ala Thr
595 600 605

Ile Ser Ala Asn Gly Lys Met Phe Gly Ser Ala Val Val Glu Asn Gly
610 615 620

Thr Ala Thr Ile Asn Leu Thr Gly Leu Thr Asn Glu Ser Thr Leu Thr
625 630 635 640

Leu Thr Val Val Gly Tyr Asn Lys Glu Thr Val Ile Lys Thr Ile Asn
645 650 655

Thr Asn Gly Glu Pro Asn Pro Tyr Gln Pro Val Ser Asn Leu Thr Ala
660 665 670

Thr Thr Gln Gly Gln Lys Val Thr Leu Lys Trp Asp Ala Pro Ser Thr
675 680 685

Lys Thr Asn Ala Thr Thr Asn Thr Ala Arg Ser Val Asp Gly Ile Arg
690 695 700

80

Glu Leu Val Leu Leu Ser Val Ser Asp Ala Pro Glu Leu Leu Arg Ser
705 710 715 720

Gly Gln Ala Glu Ile Val Leu Glu Ala His Asp Val Trp Asn Asp Gly
725 730 735

Ser Gly Tyr Gln Ile Leu Leu Asp Ala Asp His Asp Gln Tyr Gly Gln
740 745 750

Val Ile Pro Ser Asp Thr His Thr Leu Trp Pro Asn Cys Ser Val Pro
755 760 765

Ala Asn Leu Phe Ala Pro Phe Glu Tyr Thr Val Pro Glu Asn Ala Asp
770 775 780

Pro Ser Cys Ser Pro Thr Asn Met Ile Met Asp Gly Thr Ala Ser Val
785 790 795 800

Asn Ile Pro Ala Gly Thr Tyr Asp Phe Ala Ile Ala Ala Pro Gln Ala
805 810 815

Asn Ala Lys Ile Trp Ile Ala Gly Gln Gly Pro Thr Lys Glu Asp Asp
820 825 830

Tyr Val Phe Glu Ala Gly Lys Tyr His Phe Leu Met Lys Lys Met
835 840 845

Gly Ser Gly Asp Gly Thr Glu Leu Thr Ile Ser Glu Gly Gly Ser
850 855 860

Asp Tyr Thr Tyr Thr Val Tyr Arg Asp Gly Thr Lys Ile Lys Glu Gly
865 870 875 880

Leu Thr Ala Thr Thr Phe Glu Glu Asp Gly Val Ala Thr Gly Asn His
885 890 895

Glu Tyr Cys Val Glu Val Lys Tyr Thr Ala Gly Val Ser Pro Lys Val
900 905 910

Cys Lys Asp Val Thr Val Glu Gly Ser Asn Glu Phe Ala Pro Val Gln
915 920 925

Asn Leu Thr Gly Ser Ala Val Gly Gln Lys Val Thr Leu Lys Trp Asp
930 935 940

Ala Pro Asn Gly Thr Pro Asn Pro Asn Pro Asn Pro Asn Pro Asn Pro
945 950 955 960

Gly Thr Thr Thr Leu Ser Glu Ser Phe Glu Asn Gly Ile Pro Ala Ser
965 970 975

Trp Lys Thr Ile Asp Ala Asp Gly Asp Gly His Gly Trp Lys Pro Gly
980 985 990

Asn Ala Pro Gly Ile Ala Gly Tyr Asn Ser Asn Gly Cys Val Tyr Ser
995 1000 1005

Glu Ser Phe Gly Leu Gly Gly Ile Gly Val Leu Thr Pro Asp Asn Tyr
1010 1015 1020

Leu Ile Thr Pro Ala Leu Asp Leu Pro Asn Gly Gly Lys Leu Thr Phe
1025 1030 1035 1040

Trp Val Cys Ala Gln Asp Ala Asn Tyr Ala Ser Glu His Tyr Ala Val
1045 1050 1055

Tyr Ala Ser Ser Thr Gly Asn Asp Ala Ser Asn Phe Thr Asn Ala Leu
1060 1065 1070

Leu Glu Glu Thr Ile Thr Ala Lys Gly Val Arg Ser Pro Glu Ala Ile
1075 1080 1085

Arg Gly Arg Ile Gln Gly Thr Trp Arg Gln Lys Thr Val Asp Leu Pro
1090 1095 1100

Ala Gly Thr Lys Tyr Val Ala Phe Arg His Phe Gln Ser Thr Asp Met
1105 1110 1115 1120

Phe Tyr Ile Asp Leu Asp Glu Val Glu Ile Lys Ala Asn Gly Lys Arg
1125 1130 1135

Ala Asp Phe Thr Glu Thr Phe Glu Ser Ser Thr His Gly Glu Ala Pro
1140 1145 1150

Ala Glu Trp Thr Thr Ile Asp Ala Asp Gly Asp Gly Gln Gly Trp Leu
1155 1160 1165

Cys Leu Ser Ser Gly Gln Leu Asp Trp Leu Thr Ala His Gly Gly Thr
1170 1175 1180

Asn Val Val Ala Ser Phe Ser Trp Asn Gly Met Ala Leu Asn Pro Asp
1185 1190 1195 1200

Asn Tyr Leu Ile Ser Lys Asp Val Thr Gly Ala Thr Lys Val Lys Tyr
1205 1210 1215

Tyr Tyr Ala Val Asn Asp Gly Phe Pro Gly Asp His Tyr Ala Val Met
1220 1225 1230

Ile Ser Lys Thr Gly Thr Asn Ala Gly Asp Phe Thr Val Val Phe Glu
1235 1240 1245

Glu Thr Pro Asn Gly Ile Asn Lys Gly Gly Ala Arg Phe Gly Leu Ser
1250 1255 1260

Thr Glu Ala Asn Gly Ala Lys Pro Gln Ser Val Trp Ile Glu Arg Thr
1265 1270 1275 1280

Val Asp Leu Pro Ala Gly Thr Lys Tyr Val Ala Phe Arg His Tyr Asn
1285 1290 1295

Cys Ser Asp Leu Asn Tyr Ile Leu Leu Asp Asp Ile Gln Phe Thr Met
1300 1305 1310

Gly Gly Ser Pro Thr Pro Thr Asp Tyr Thr Tyr Thr Val Tyr Arg Asp
1315 1320 1325

Gly Thr Lys Ile Lys Glu Gly Leu Thr Glu Thr Thr Phe Glu Glu Asp
1330 1335 1340

Gly Val Ala Thr Gly Asn His Glu Tyr Cys Val Glu Val Lys Tyr Thr
1345 1350 1355 1360

Ala Gly Val Ser Pro Lys Glu Cys Val Asn Val Thr Ile Asn Pro Thr
1365 1370 1375

Gln Phe Asn Pro Val Lys Asn Leu Lys Ala Gln Pro Asp Gly Gly Asp
1380 1385 1390

Val Val Leu Lys Trp Glu Ala Pro Ser Ala Lys Lys Thr Glu Gly Ser
1395 1400 1405

Arg Glu Val Lys Arg Ile Gly Asp Gly Leu Phe Val Thr Ile Glu Pro
1410 1415 1420

Ala Asn Asp Val Arg Ala Asn Glu Ala Lys Val Val Leu Ala Ala Asp
1425 1430 1435 1440

Asn Val Trp Gly Asp Asn Thr Gly Tyr Gln Phe Leu Leu Asp Ala Asp
1445 1450 1455

His Asn Thr Phe Gly Ser Val Ile Pro Ala Thr Gly Pro Leu Phe Thr
1460 1465 1470

Gly Thr Ala Ser Ser Asn Leu Tyr Ser Ala Asn Phe Glu Tyr Leu Ile
1475 1480 1485

Pro Ala Asn Ala Asp Pro Val Val Thr Thr Gln Asn Ile Ile Val Thr.
1490 1495 1500

Gly Gln Gly Glu Val Val Ile Pro Gly Gly Val Tyr Asp Tyr Cys Ile
1505 1510 1515 1520

Thr Asn Pro Glu Pro Ala Ser Gly Lys Met Trp Ile Ala Gly Asp Gly
1525 1530 1535

Gly Asn Gln Pro Ala Arg Tyr Asp Asp Phe Thr Phe Glu Ala Gly Lys
1540 1545 1550

Lys Tyr Thr Phe Thr Met Arg Arg Ala Gly Met Gly Asp Gly Thr Asp
1555 1560 1565

Met Glu Val Glu Asp Asp Ser Pro Ala Ser Tyr Thr Tyr Thr Val Tyr
1570 1575 1580

Arg Asp Gly Thr Lys Ile Lys Glu Gly Leu Thr Glu Thr Thr Tyr Arg
1585 1590 1595 1600

Asp Ala Gly Met Ser Ala Gln Ser His Glu Tyr Cys Val Glu Val Lys
1605 1610 1615

Tyr Ala Ala Gly Val Ser Pro Lys Val Cys Val Asp Tyr Ile Pro Asp.
1620 1625 1630

Gly Val Ala Asp Val Thr Ala Gln Lys Pro Tyr Thr Leu Thr Val Val
1635 1640 1645

Gly Lys Thr Ile Thr Val Thr Cys Gln Gly Glu Ala Met Ile Tyr Asp
1650 1655 1660

Met Asn Gly Arg Arg Leu Ala Ala Gly Arg Asn Thr Val Val Tyr Thr
1665 1670 1675 1680

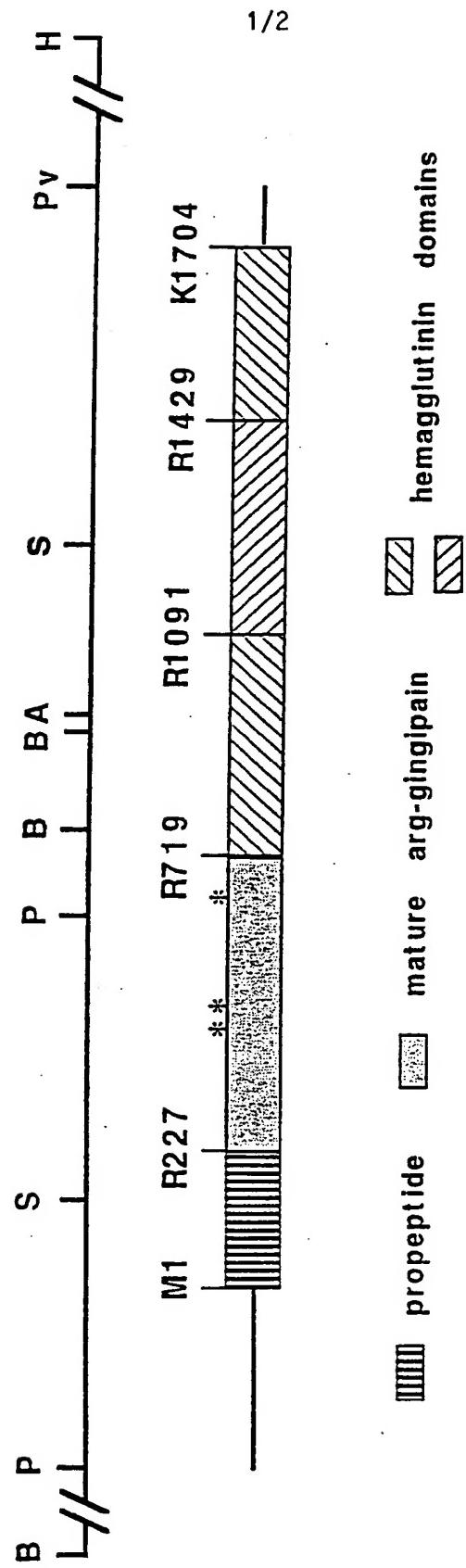
Ala Gln Gly Gly Tyr Tyr Ala Val Met Val Val Val Asp Gly Lys Ser
1685 1690 1695

Tyr Val Glu Lys Leu Ala Val Lys
1700

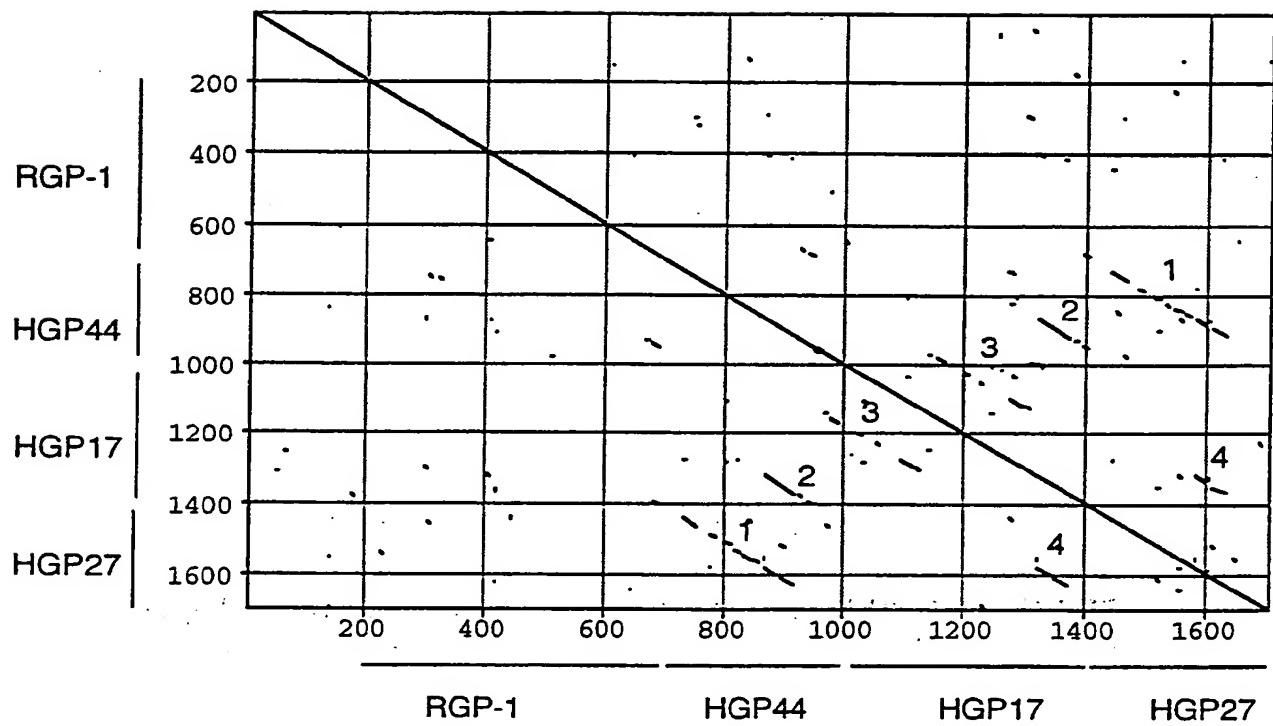
WE CLAIM:

- 1
- 1 1. A recombinant DNA molecule comprising a nucleotide sequence
2 encoding an Arg-gingipain protein having an amino acid
3 sequence selected from group consisting of sequences as
4 given in SEQ ID NO:4 from amino acid 1 through amino acid
5 510, SEQ ID NO:11 from amino acid 228 through amino acid
6 719, and an amino acid sequence having at least about 85%
7 amino acid sequence identity with a sequence as given in
8 SEQ ID NO:11 from amino acid 228 to amino acid 719.
- 1
- 2 2. The recombinant DNA molecule of claim 1, wherein said
3 nucleotide sequence is as given in one of SEQ ID NO:3 from
4 nucleotide 1630 through nucleotide 3105 and SEQ ID NO:10
from nucleotide 1630 through nucleotide 3105.
- 1
- 2 3. A recombinant DNA molecule comprising a nucleic acid
3 portion encoding a high molecular weight Arg-gingipain
4 comprising an enzymatically active protease component and
a hemagglutinin component.
- 1
- 2 4. The recombinant DNA molecule of claim 3 wherein said
3 encoded high molecular weight Arg-gingipain has an
4 enzymatically active protease component having an amino
5 acid sequence as given in one of SEQ ID NO:4 from amino
6 acid 1 to amino acid 510 and SEQ ID NO:11 from amino acid
228 to amino acid 719.
- 1
- 2 5. The recombinant DNA molecule of claim 4 herein said high
3 molecular weight Arg-gingipain has an enzymatically active
4 protease component having an amino acid sequence as given
5 in SEQ ID NO:11 from amino acid 228 to amino acid 719 and
6 a hemagglutinin component having an amino acid sequence
selected from the group consisting from amino acid 720 to
7 amino acid 1091, from amino acid 1092 to amino acid 1429
8 and from amino acid 1430-1704, each as given in SEQ ID NO:11.

- 1 6. The recombinant DNA molecule of claim 4 wherein said mature
2 enzymatically active protease component is encoded by a
3 nucleotide sequence as given in one of SEQ ID NO:3 from
4 nucleotide 1630 to nucleotide 3105, in SEQ ID NO:10 from
5 nucleotide 1630 to nucleotide 3105 or a nucleotide sequence
6 having at least 70% homology to one of said sequences.
- 1 7. The recombinant DNA molecule of claim 1 wherein said Arg-
2 gingipain is encoded within a nucleotide sequence as given
3 in SEQ ID NO:10 from nucleotide 949-6063, or a nucleotide
4 sequence having at least about 70% sequence homology
5 thereto.
- 1 8. The recombinant DNA molecule of claim 7 wherein said Arg-
2 gingipain is expressed as a prepolyprotein having an amino
3 acid sequence as given in SEQ ID NO:11.
- 1 9. The recombinant DNA molecule of claim 8 wherein the
2 nucleotide sequence encoding said polyprotein is as given
3 in SEQ ID NO:10 from nucleotide 949 to nucleotide 6063.



2/2



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/10283

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :435/69.1, 195, 212, 213, 220, 320.1; 536/22.1, 23.1, 23.2, 23.7

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 195, 212, 213, 220, 320.1; 536/22.1, 23.1, 23.2, 23.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 267, No. 26, issued 15 September 1992, Chen et al., "Purification and Characterization of a 50-kD Cysteine Proteinase (Gingipain) from <i>Porphyromonas gingivalis</i> ", pages 18896-18901, see entire document.	1-9
Y	FASEB JOURNAL ABSTRACT, Vol. 5, No. 4, issued 1991, Potempa et al., "Purification and Characterization of a 50 kDa Cysteine Proteinase of <i>Porphyromonas gingivalis</i> ", page A829, abstract no. 2667, see entire abstract.	1-9
Y	SCIENCE, Vol. 239, issued 11 March 1988, Lee et al., "Generation of cDNA Probes Directed by Amino Acid Sequence: Cloning of Urate Oxidase", pages 1288-1291, see entire document.	1-9

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	&	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

18 DECEMBER 1994

Date of mailing of the international search report

05 JAN 1995

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10283

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07H 17/00, 19/00, 21/00; C12N 9/14, 9/48, 9/52, 9/76, 15/00; C12P 21/06

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE, STN, BIOSIS

search terms: bacteroides gingivalis, porphyromonas gingivalis, gingipain, arg gingipain, proteinase?, arg, gingipain-1, gingipain-2